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International application number: PCT/US05/015764

International filing date: 06 May 2005 (06.05.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/569,370
Filing date: 06 May 2004 (06.05.2004)

Date of receipt at the International Bureau: 20 June 2005 (20.06.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
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APPLICATION NUMBER: *60/569,370*
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Trent Russell	Northen	Tempe, Arizona
Neal Walter	Woodbury	Tempe, Arizona

Additional inventors are being named on the ___ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

LIGHT DIRECTED SOLID PHASE SYNTHESIS ON PATTERNED POLYMERS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 54
☐ Drawing(s) *Number of Sheets*
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify) Cover sheet; Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees
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☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Robert D. Atkins

Date 05/6/04

TYPED or PRINTED NAME Robert D. Atkins

REGISTRATION NO. *(if appropriate)* 34,288

Docket Number: 112624.00138

TELEPHONE (602) 229-5311

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Patent Application, Commissioner for Patents, Alexandria, VA 22313-1450.

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MARITZA O'NEILL

CERTIFICATE OF MAILING PURSUANT TO 37 C.F.R. 1.10

Applicant: Northen *et al.*

Filed: May 6, 2004

Title: *LIGHT DIRECTED SOLID PHASE SYNTHESIS ON PATTERNED POLYMERS*

Docket No.: 112624.00138

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Type of Filing:

1) Provisional Application For Patent Cover Sheet
2) Specification (54 pages, plus cover sheet)
3) Return postcard

PATENT

PROVISIONAL APPLICATION

of

TRENT RUSSELL NORTHEN
NEAL WALTER WOODBURY

For

UNITED STATES LETTERS PATENT

on

LIGHT DIRECTED SOLID PHASE SYNTHESIS ON PATTERNED POLYMERS

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Attorney Docket No.: 112624.00138

(Include additional names and addresses on a separate sheet.)

II. DESCRIPTIVE TITLE OF INVENTION

___ Light directed solid phase synthesis on patterned polymers

III. GRANT/CONTACT (If any):

Sponsor(s) none Award Number _____

Principal Investigator: n/a ORSPA Acct Number _____

IV. LAB/DEPARTMENT WHERE DEVELOPED:

Woodbury lab, Department of Chemistry and Biochemistry, ASU

V. DESCRIPTION OF INVENTION:

A. This invention is a(n): _____x_____process _____chemical compound

_____electronic circuit _____mixture of chemical compounds

_____apparatus _____therapeutic method _____other

(describe)

B. State, as fully as possible, what the invention is, including: materials or components used; operative and preferred ranges of process parameters and concentrations of chemical compounds; and foreseeable uses of the invention.

___ This invention combines three existing technologies: 1. photopolymers (photoresist), 2. photolabile protective groups, and 3. solid phase synthesis. The combination of these three existing technologies allows for the construction of three dimensional surfaces and devices that have tailored chemical functionality in spatially defined areas. The substrate can be any polymer that is in the appropriate three dimensional form having groups that can be derivatized in a way that can then be protected with photolabile protective groups. Photolabile protective groups can

three dimensional form having groups that can be derivatized in a way that can then be protected with photolabile protective groups. Photolabile protective groups can include any group that can be removed with light or activated by light in a way to expose or react with a material introduced in solution. Ways of patterning the polymer may include photopolymerization, thermal polymerization, or contact stamping. Ways of removing the photoprotective group include using a scanning laser system, micromirror array, or photolithographic method. Compounds that can be attached to this surface can be almost anything that will reacted with the given functionality exposed upon removal of the photolabile protective groups. It is possible to use both single and multiphoton excitation of the polymer and protective group to generate the spatial features. Sequential steps of removing the photolabile protective group and coupling new materials with the protective group blocking the appropriate reactive groups, can be used to generate complex patterns of functionalized polymer surfaces. In the short term these would be useful for enhanced DNA and Peptide microarrays, longer term these could be used for things as diverse as drug delivery systems, sensors, and artificial organs.

This includes a vast number of materials and methods. Work to date has been with acrylates and methacrylates including ones with reactive side chains (epoxy) that can be functionalized with diamines to yield aminated surfaces. These polymer surfaces have been three dimensionally patterned from photoreactive monomer/polymer solutions using a scanning laser system on top of a methacrylate functionalized glass surface. They have been protected with the nitroveratryloxycarbonyl (NVOC) photolabile protecting group (there are several protecting groups for amines and hydroxyls based on the general class of nitrobenzene compounds, though there are other classes of protective groups that can be used). This protective group has been removed using a scanning laser system. Detection of the deprotective areas has been done using fluorescence from a reactive dye that selectively reacts with the exposed amine groups.

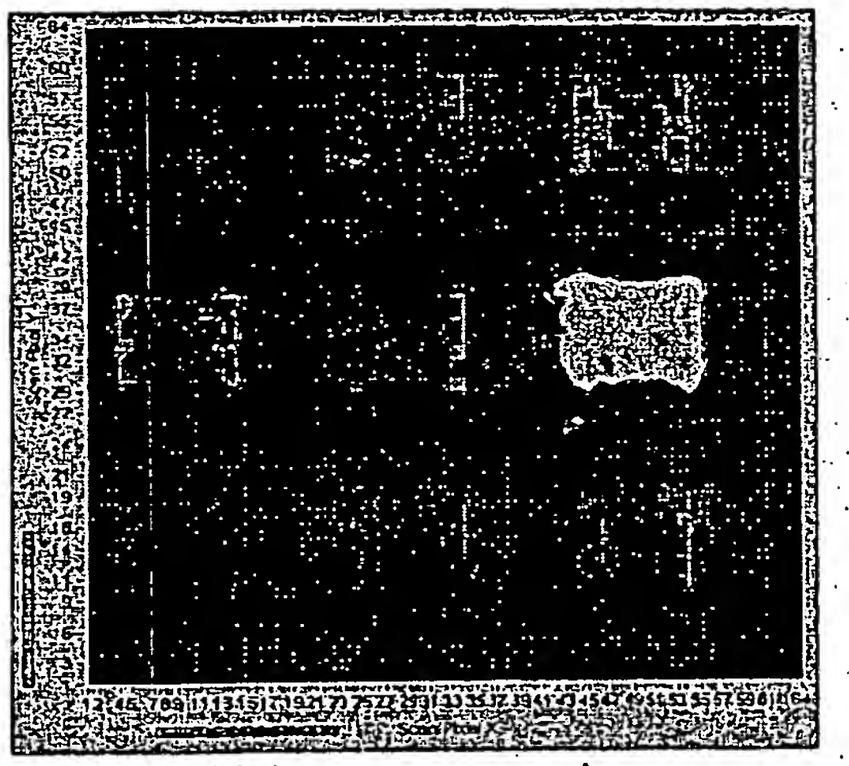
C. Records Supporting Invention: Identify records which establish dates of conception and reduction to practice, including identity of person who prepared record and its present location. Attach copies if possible. Note additional supporting evidence. If the invention or a significant aspect of the invention is not supported by written records, briefly describe how the date of invention can be established and identify earliest written record.

A glass cover slide was cleaned for 15 min at RT with 60/40 sulfuric acid/hydrogen peroxide, placed in 10% sodium hydroxide at 70 C for 3 min, placed in 1% HCl at RT for 1 min, between each step it was soaked in nanopure water for 3 minutes. A solution of of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was made and mixed for 10 minutes, the slide was then added and left to react at RT for 15 minutes with gentile agitation. This slide was soaked in isopropyl alcohol for 3 min then nanopure water for 1 min then placed in a 100 C oven for 5 minutes after which the oven was

turned off and nitrogen was blown through for 1 hr. The slide was stored under nitrogen until it was used.

A blend of methacrylate monomers and photoinitiator (900 uL trimethylolpropane trimethacrylate, 100uL glycidyl methacrylate, 10 mg azobisisobutyronitrile) was prepared and nitrogen was bubbled through for 15 minutes before it was injected into a Focht cell (Bioptechs inc. Butler, PA) that had been flushed with argon. This cell was then mounted onto a Prior scientific microscope stage on a Nikon microscope and illumiated through a 40x 0.75 NA objective with 370nm light from a Ti:Saphire laser modulated by a Conoptics modulator (shutter). The system was controlled via National instruments board and in house software. A pattern of nine square 500 micron features was patterned with 20 micron spacing at 99% scan rate with 100 microwatts of power input into the microscope ~ 25 microwatts output from objective.

Unpolymerized monomer was removed by washing with diethylether. The chamber was then filled with a 10 % 1,4-Bis(3-aminopropoxy)butane solution in dimethylformamide (DMF) for 15 minutes at room temperature. The chamber was rinse with DMF and then a solution of 14mg NVOC, 10 uL diisopropylethylamine (DIPEA), and 500 uL DMF was added and allowed to react for 35 minutes. The system was again rinsed with DMF and filled with dioxane. Two squares were scanned on the same laser system described above, with 10 scan lines per feature at 10% scan rate, one was scanned with 5 mW input power and the other 1 mW input power.



The chamber was then filled with a solution of 10mg dansyl chloride, 10 uL DIPEA, and 500 uL DMF and allowed to react for 15 minutes. The chamber was then rinsed with DMF to remove the excess dye and imaged on the same scanning laser system, where the 40x objective had been replaced by a 10x 0.30 NA objective. Fluorescence was collected via an avalanch photodiode which was processed by a Becker and Hickl Time-Correlated single photon counting module.

The data shows that the feature patterned with 5 mW (white) is significantly brighter than the other features (black/gray):

D. Fill in the following dates:

1. Conception Early March

2. First disclosure to another In early to mid-march I told a lab tech and undergraduate who work with/for me of the concept and told them that I was going to be focused on it from that point forward

3. First written record 03/11/2004

4. First experiment demonstrating the invention 04/14/2004

E. This invention can be used as Enhanced microarray technology where the signal is orders of magnitude larger making it easy to detect binding events (less sensitive instrumentation). Immediate application of this would be for **DNA microarrays.** This could also be used for the synthesis of large arrays of heteropolymers that could be used in **drug development, molecular evolution, or sensor development**. Hence, Lab on a chip analytical applications, analytical devices, or **microsensor** applications. Using multiphoton detection three dimensional surfaces can be decorated with functional groups using this method, these could be cell recognition factors, allowing the construction of complex three dimensional cellular arrays which could be used as bioreactors or **artificial organs**. These three dimensional surfaces could also be functionalized to form novel **biomaterials** or **drug delivery** systems.

F. The problem which this invention solves is
Low signal from microarrays which very sensitive equipment to detect. inability to construct three dimensionally dimensionally functionalized materials with a high level of spatial control. Difficulty in screening and or encoding combinatorial libraries.

G. The closest prior art is
Frechet Jean M.J. et al, Journal of Polymer Science Part A, 2002, Vol 40, 755-769 and Macromolecules 2003, 36, 1677-1684, used photolithography to prepare monolithic polymers in a spatially defined manner in glass capillaries.

Fodor et al, Science, vol 251, 767-773. Used photolithography in combination with a nitroveratryloxycarbonyl (NVOC) photolabile protective group to synthesize arrays of peptides on a glass substrate.

Satoshi Kawata et al, Nature 2001 vol 412 page 697-698. has created submicron objects using photopolymers in conjunction with two photon excitation.
Shoji Maruo et all, Sensors and Actuators A vol 100, 70-76. Has used single photon excitation to create 430nm photopolymer features.

H. This invention differs from the closest prior art in that

Frechet used a very different chemistry. He uses free radical polymerization developed by (Ranby, B.) to graft polymers onto polymer surfaces. Therefore he can 'grow' a polymer on the surface of another polymer in a two dimensionally defined way. However, he does not have precise control on the products and can not synthesize heteropolymers with defined sequence in a spatially defined way. Where we propose spatially defined step wise synthesis; protect, deprotect, couple protected monomer/polymer, repeat until a complex structure is created. His method does not have the possibility of doing three dimensional controlled surface functionalization.

Fodor uses glass rather than a patterned polymer surfaces, he does use the photolabile protective in a repeated cycle of coupling and deprotection steps. He is limited to two dimensional patterning.

Kawata and Maruo have constructed three dimensional polymer devices using scanning microscopes but have not, to our knowledge done and synthesis or functionalization on the polymer surfaces.

I. This invention provides the following advantages:

The ability to construct spatially defined functionalized polymer structures of great diversity in two and three dimensions. The larger surface area of the polymer vs. glass increases the signal and potentially the sensitivity vs. conventional microarrays.

Patent Disclosure 04/16/2004

Inventors:

Trent Russell Northen, Neal Walter Woodbury, Sudhir Gudala.
Department of Chemistry and Biochemistry, Arizona State University

Date of invention: 03/11/2004
Date of proof of principle: 04/14/2004

Title: Light directed solid phase synthesis on patterned photopolymers.

Overview:

A new technology has been developed by the inventors that has potential to offer significant advantages over existing microarray technology with long term applications to sensor development, drug development, drug delivery, molecular evolution, and biomaterials.

This invention hinges on the combination of three existing technologies: 1. photopolymers (photoresist – plastic materials that can be patterned in three dimensions with light), 2. photolabile protective groups (these enable the patterned formation of specific chemical bonds in three dimensional space), and 3. solid phase synthesis (the process of generating complex heteropolymers such as, but not limited to, DNA and protein with known sequences on solid surfaces in a completely automated fashion). The combination of these three existing technologies allows for the construction of three dimensional arrays and devices that have tailored chemical functionality. Because of the three dimensional aspect of the photopatterning, an increase of several orders of magnitude in signal strength from, for example, DNA can be obtained as well as dramatic increases in the array density (both two and three dimensional arrays). It has the potential to change the paradigm for the current technology of both DNA and peptide arrays. In the long term this technology could be used to make sensors, combinatorial chemistry platforms, drug delivery devices, biomaterials, and even serve as the scaffold for generating artificial organs.

Huge Opportunity:

"The total biochip market size in 2001 is about $740 million and may more than triple in revenues, to about $2.47 billion in 2006" (Technology, Strategic Alliance, Patent Dispute and Market Update – 2002).

Briefly, light is used to make little arrays of solid phase synthesis polymer (which is basically the same thing as photoresist used the electronics industry) on a glass substrate. This polymer can be made in such a way that it is either porous or has a very rough surface (very large surface area) that is covered with reactive chemical groups (for example, primary amines). The reactive groups are then made unreactive by adding a

special blocking group that is photolabile (can be removed by exposure to light). Now, individual polymer elements of the array can be illuminated making them reactive in a patterned fashion and then reacted with materials of interest. One can then add specific molecules only to the elements that have been illuminated. If the molecules added themselves have reactive groups that are blocked by a photolabile blocking group, the process can be continued in layers, building up specific heteropolymers in a patterned fashion. The photolabile blocking group chemistry is the same as what has been used by Affymetrix and other companies to make DNA arrays. The difference is that instead of a monolayer of DNA (or peptide or other heteropolymer) on a surface, one has a much larger number of molecules in the same 2-dimensional element because of the third dimension afforded by basing the array on porous or rough-surfaced polymer elements. This greatly amplifies the signal, making it much easier to detect (the fluorescence from dye reacted directly with the polymer elements is easy to see by eye).

Time Imperative:

There is a group at Berkeley that has recently (2003) published very relevant work. That work does not yet include combining patterning of chemicals on polymer elements using photolabile blocking groups, but they have all of the technology available to them if they were to decide to go in this direction. It is critical that we move quickly to secure our rights to this potentially very significant invention.

Patent discloser:

Inventors: Trent Northen, Sudhir Gudala & Neal Woodbury
Date: 04.16.2004

Title: Light directed solid phase synthesis on patterned photopolymers.

Summary: A general method has been developed to create polymer features and modifying the functionality of the polymer in a spatially resolved manner using photolabile protecting groups to control the addition of desired functional groups.

This invention combines several existing technologies in a novel and useful way. The relevant technologies include: Solid phase synthesis, light directed polymerization, and light directed polymer synthesis.

Disclosed is the method of making polymer structures that have spatially defined chemical features through 1.) photopolymerization to form polymer structures 2.) protection of functional polymer features with photolabile protective group(s) 3.) photodeprotection of desired polymer features, 4.) reaction of deprotected reactive sites with desired reactive chemical species, and 5.) if desired repetition of these steps to form complex functional features.

The features of the polymer and photodeprotected region can be controlled through the modulation of the irradiating light. Small features (~1 micrometer) are generated using high numerical aperture objective lenses and even smaller features can be made using multiphase excitation (50-1000nm) or classical masking methods used in the semiconductor industry.

Background:

Photopolymers:

Photopolymer photo resists are well known and have been used for many years to create small features in the microelectronics industry. More recently they have been used in rapid prototyping or stereo lithography:

- Jan F. Rabek Mechanisms of photophysical processes and photochemical reactions in polymers 1987 John Wiley and Sons Ltd.

Most recently photopolymers have been used in conjunction with high numerical aperture lenses and multiphoton excitation to create very small three dimensional objects.

- Satoshi Kawata and coworkers, Advanced Materials 2003 vol 15, 2011-2014 has used single and multi photon interferential patterning to generate features as small as 50 nm.
- Satoshi Kawata et al, Nature 2001 vol 412 page 697-698. has created submicron objects using photopolymers in conjunction with two photon excitation.

- Shoji Maruo et all, Sensors and Actuators A vol 100, 70-76. Has used single photon excitation to create 430nm photopolymer features.

Spatially resolved biopolymer synthesis is well known and has been used for years to synthesize DNA arrays on glass substrates:

- Fodor et al, Science, vol 251, 767-773. Used photolithography in combination with a nitroveratryloxycarbonyl (NVOC) photolabile protective group to synthesize arrays of peptides on a glass substrate.
- McGall et al, JACS, 1997 vol. 119 page 5081-5090. Used photolithography in combination with the 5'-((α-methyl-2-nitropiperonyloxy)carbonyl) (MeNPOC) to synthesize DNA arrays on glass substrates.
- Michael R. Sussman and co workers, Nature Biotechnology, vol 117, 974-978 used micromirror arrays in conjunction with the MeNPOC protective group to synthesize DNA microarrays.
- Gerard Cagney and coworkers, Nature Biotechnology, vol 18, 2000, 393-397 discusses different applications of protein and peptide arrays.

Solid Phase Synthesis (SPS) is well know and is a method of choice for synthesizing biopolymers (peptides, DNA, etc):

- Merrifield R.B., JACS 1963 Vol 85, 2149-2154 first synthesized a tetrapeptide on a solid resin particle (polystyrene).
- Barany G. et al, JACS 1996, vol 118, 7083-7093 has synthesized a solid phase resin that swells in both water and organic solvents using various methacrylate resins.
- Frechet Jean M.J. et al, Journal of Polymer Science Part A, 2002, Vol 40, 755-769 and Macromolecules 2003, 36, 1677-1684, used photolithography to prepare monolithic polymers in a spatially defined manner in glass capillaries.

Solid phase synthesis techniques have been used to generate combinatorial libraries. These methods have become common to the art, they typically include, dividing the SPS beads into pools after each synthesis step to generate large libraries of peptides. The peptide can be screened and cleaved from the bead can be encoded with some sort of tag for identification

- Lam Kit S. Chem. Reviews 1997, 411-448 this "One-Bead-One-Compound" method.

Photolabile protecting groups:

- Bochet Christain G., Journal of the chemical society, Perkin Transactions 1 2002 vol 2 125-142. Reviews the most common photolabile protective groups.

Biomaterials:

- Langer R. et al, Nature, vol 428 2004 487-492. Reviews biomaterial technology.
- Fisher J.P. et al Annu. Rev. Mater. Res. Vol 31 2001 171-181 describes photoinitiated polymerization and polymer crosslinking for biomaterial synthesis.

One of the significant disadvantages to the existing methods for spatially resolved biopolymer synthesis (Fodor, McGall, and Sussman) is the limited number of reactive sites available on the glass surface (McGall estimates 10-30 picomole/sq-cm). Characterization of reaction products becomes very difficult, requiring sensitive techniques and instruments, for example the most common technique, which is well known to one skilled in the art, for using and characterizing DNA arrays the hybridization of fluorescence probes and use of a scanning epifluorescent microscope to detect these probes. In the case of DNA since a fluorescently labeled complimentary strand can be made for each array element, it would in theory, be possible to characterize any DNA microarry with this technique under the appropriate hybridization conditions.

Since peptides cannot be probed in this same way, due to the non-complimentarity of their structures, other more complicated systems are used. Most commonly, the use of antibody systems in which one antibody is labeled with a fluorescent dye and one antibody (could be the same) is specific for the peptide sequence to be probed (Fodor). This is useful for a proof of principle, but would be impractical for probing large number of peptides.

Even though techniques have evolved to allow the synthesis and screening of libraries using SPS techniques (SPS) screening of the beads is complex.

Polymer structures can be functionalized using this method with cell recognition factors or binding factors such as.....these can be added/deprotected in a spatially controlled manner to create tailored structures. This invention combines the benefits of the array format, large number of reactive sites available in porous solid phase synthesis resin and the ability to form polymer structures using photopolymers. Resulting in larger signals, improved contrast ratios, and better applicability of analytical characterization techniques than existing microarray methods. Since the array is positionally encoded, it is easier to screen and probe than the split pool methods.

These are simply a result of having larger number of sites then on the glass substrate. So that the fluorescence signal is larger when using fluorescent probes or the amount of product produced in a given is large enough to be able to characterize products cleaved off the resin by common analytical techniques such as mass spectroscopy, FTIR, etc.

Further, the array format spatially encodes the peptides so that it is easier to probe than the split pool libraries. These arrays can be probed with analyte for sensor development, drug discovery, or for cell adhesion in biomaterial development.

This invention allows the generation of small three dimensional structures that can be functionalized in spatially defined ways for the construction of sensors, catalysis, biomaterials, drug delivery, molecular evolution, etc.

<u>Summary of the invention:</u>

The system is composed of a photopolymer bearing a reactive group, photolabile protecting group(s), groups to be attached that can also contain the photolabile protective group(s), and devices for illuminating the sample and introducing/removing new reagents. Groups to be attached are not limited to single molecules but could also include macromolecules and even cells.

Polymers/monomers:

For a system where it is desired to detect fluorescence from the array it is important that the polymer system not absorb the excitation light and that it not emit at the detection wavelength. In this case any nonfluorecent nonabsorbing (at the deprotection wavelength) and nonemiting (at detection wavelength) polymer or monomer systems can be used including monomers which are polymerized or polymers that are crosslinked or both. One or more of the following: acrylate, methacrylate, urethane, epoxy, urea, cellulose monomers, protein, glycols, lactic acid, ε-caprolactone, trimethylene carbonate, N-vinylpyrrolidinone, 2,2 dimethoxy-2-phenylacetophenone, esters, DNA, RNA, ...containing side
chains.....
. or polymers of these monomers and or combinations of these monomers.

Solvents can be incorporated into these systems to modify the pore structure of the polymers. Solvents can include alcohols (methanol, ethanol, butanol, isopropanol, cyclohexanol), acetone, acetonitrile, toluene, etc.

Most prefered are methacrylates and acrylates.

Functionalization:

Polymers/monomers can themselves contain pendent reactive groups like hydroxyls, epoxy, amino, etc groups or they can be incorperated after the polymerization reaction.

Photoiniators:

Photoinitiators (adapted from JP Fouassier progress in organic coatings vol 47, 2003 16-36) can include in the general classes of initiators: halogens, halogenated organic compounds, hydrogen peroxide, alkyl hydroperoxides, cumene hydroperoxide, peroxides, benzoyl peroxide, non-ketonic peresters, ketones, quinones, polycyclic hydrocarbons, azocompounds, hydrazones, cyclic acetals, 1,3-dithiolane, soccharides, metal oxides, ion pair complexes, metal chlorides, uranium salts, metal carbonyls, metal acetylacetonates, ferrocene, metal complexes, dyes, and polymeric photoinitaiarots. More specifically radical iniators: azides like azobisisobutyronitrile and derivatives, ketones like benzophenone, thioxanthone, acridone aromatic diketones and derivatives, ketocoumarins and coumarins derivatives; dyes (e.g. xanthene dyes such as eosin (EO) or Rose Bengal (RB), thioxanthene dyes or cyanins); thioxanthones; bis-acylphosphine oxides; peresters; pyrylium and thiopyrylium salts in the presence of additives such as a perester; cationic dyes containing a borate anion; dyes/bis-imidazole derivatives/thiols; PS/chlorotriazine/additives; metallocene derivatives (such as titanocenes); dyes or ketones/metallocene derivatives/amines; cyanine dyes in the presence of additives;

dyes/bis-imidazoles; miscellaneous systems such as phenoxazones, quinolinones, phthalocyanines, squaraines, squarylium containing azulenes, novel fluorone visible light PIs, benzopyranones, rhodamines, riboflavines, RB peroxybenzoate, PISs with good photosensitivity to the near IR, camphorquinone/peroxides, pyrromethane dye, crystal violet/benzofuranone derivatives, two color sensitive systems, etc.

Colored cationic PIs (such as iron arene salts, novel aromatic sulfonium or iodonium salts) and PS/cationic PI (where PS can be hydrocarbons or ketones or metal complexes) can help to shift the absorption in the visible wavelength range.

Non-ionic photoacids and photobases for the generation of active species in photoresists technology are developed. By now, the design of colored species and proposals of PS for their decomposition remains attractive challenges.

Excited state processes of photosensitive systems for laser beams and/or conventional light sources induced polymerization reactions have been reported in recent works Typical photosensitive systems under visible lights are classified as One-component system (such as bis-acylphosphine oxides, iron arene salts; peresters, organic borates, titanocenes, iminosulfonates, oxime esters, etc. Two-component system (working, e.g. through electron transfer/proton transfer, energy transfer, photoinduced bond cleavage via electron transfer reaction, electron transfer), Three-component system (where the basic idea is to try to enhance the photosensitivity by a judicious combination of several components).

Most preferred are Azoisobutyronitrile and it's derivatives.

Photolabile protecting groups:

Photolabile protecting agents (from Bochet) can include: *o*-Nitrobenzyl alcohol derivatives, α-Ketoester derivatives, Benzophenone reduction, Photosolvolysis-related reactions, Benzyl alcohol derivatives, Benzyl alcohol derivatives, Benzoin esters, Phenacyl esters, Acylating agents, Fluorenecarboxylates, Arylamines as photo-reductors, Benzophenone as photooxidant, Photoisomerisation *trans–cis*, Cinnamyl esters, Vinylsilanes substituted. Most preferred are nitroveratryloxycarbonyl, 5′-((α-methyl-2-nitropiperonyloxy)carbonyl)

Groups to be added:

Groups to be added onto the polymer structures include, sugars, amino acids, nucleic acids, multifunctional amines, ethylene glycol, acid labile groups, base labile groups, dyes, ...and combinations of or polymers of these monomers. Sequential light directed synthesis can be used to build complex sequence specific polymers.

Most preferred groups include amino and hydroxyl groups.

Method of light modulation:

Light can be modulated (spatially patterned) using a scanning laser system composed of a laser, shutter, microscope objective and stage. In this case the stage movement and

shutter are controlled so that the shutter is only open when the stage is positioned so that the light will illuminate a desired position.

Photolithography is well know to the art but briefly it utilizes masks where light is blocked by some parts of the mask and not others. In this way the illumination reaching the sample can be controlled. Light sources typically include lamps or lasers.

Micromirror arrays are a more recent way of modulating light. By changing the angle of the mirrors in the array light can be directed towards a surface or not. In this way light from an excitation source (lamp or laser) can be selectively reflected onto desired regions of the sample to be exposed.

The preferred embodiment is either a micromirror array or scanning laser system

Substrate: Substrates can include glass, quartz, silicon oxide or other metal oxide surfaces, polymers bearing reactive groups. It is not necessary that they be transparent since illumination can be from above. In the case of glass, quartz, and silicon oxide these surfaces can be modified to react with the polymer for a covalent linkage, though this may not be desirable or necessary in all cases since intermolecular attractive forces can be used to 'glue' the features to the substrate. Where modification is desirable silanes common to the art can be used, the most common being aminopropyl triethoxysilane or 3-(trimethoxysilyl)propyl methacrylate.

The preferred embodiment is glass cleaned with acid and base as described in McGall JACS 1997 and functionalized from a 1% solution of 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5% water.

System for introducing reagents: Systems for introducing and removing reagents include an optical flow cell coupled with manual or automated introduction and removal of reagents. Wells or plates where reagents are introduced manually or by automation. Automation is provided by machines such as peptide synthesizers that are designed to introduce and remove reagents.

Analytical Techniques:

Array elements can be probed in situ through various spectroscopic techniques including fluorescence, absorption, infrared spectroscopy, raman spectroscopy, nonlinear spectroscopy, and surface plasmon resonance or elements can be removed from the surface through the use of labile linkages between the coupled material and the polymer. Thus the material can be cleaved and a host of analytical techniques can be used including HPLC, NMR, Mass spectrometry, capillary electrophoresis.

Most preferred include fluorescence detection of hybridized, bound, or covalently linked probes or groups, infrared spectroscopy, and mass spectroscopy of cleaved materials.

Example 1:

A glass cover slide was cleaned for 15 min at RT with 60/40 sulfuric acid/hydrogen peroxide, placed in 10% sodium hydroxide at 70 C for 3 min, placed in 1% HCl at RT for 1 min, between each step it was soaked in nanopure water for 3 minutes. A solution of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was made and mixed for 10 minutes, the slide was then added and left to react at RT for 15 minutes with gentile agitation. This slide was soaked in isopropyl alcohol for 3 min then nanopure water for 1 min then placed in a 100 C oven for 5 minutes after which the oven was turned off and nitrogen was blown through for 1 hr. The slide was stored under nitrogen until it was used.

A blend of methacrylate monomers and photoinitiator (900 uL trimethylolpropane trimethacrylate, 100uL glycidyl methacrylate, 10 mg azobisisobutyronitrile) was prepared and nitrogen was bubbled through for 15 minutes before it was injected into a Focht cell (Bioptechs inc. Butler, PA) that had been flushed with argon. The above slide was mounted in the flow cell. This cell was then mounted onto a Prior scientific microscope stage on a Nikon microscope and illumiated through a 40x 0.75 NA objective with 370nm light from a Ti:Saphire laser modulated by a Conoptics modulator (shutter). The system was controlled via National instruments board and in house software. A pattern of nine square 500 micron features was patterned with 20 micron resolution at 99% scan rate with 100 microwatts of power input into the microscope ~ 25 microwatts output from objective.

Unpolymerized monomer was removed by washing with diethylether. The chamber was then filled with a 10 % 1,4-Bis(3-aminopropoxy)butane solution in dimethylformamide (DMF) for 15 minutes at room temperature. The chamber was rinse with DMF and then a solution of 14mg NVOC, 10 uL diisopropylethylamine (DIPEA), and 500 uL DMF was added and allowed to react for 35 minutes. The system was again rinsed with DMF and filled with dioxane. Two squares were scanned on the same laser system described above, with 10 scan lines per feature at 10% scan rate, one was scanned with 5 mW input power and the other 1 mW input power.

The chamber was then filled with a solution of 10mg dansyl chloride, 10 uL DIPEA, and 500 uL DMF and allowed to react for 15 minutes. The chamber was then rinsed with DMF to remove the excess dye and imaged on the same scanning laser system, where the 40x objective had been replaced by a 10x 0.30 NA objective. Fluorescence was collected via an avalanch photodiode which was processed by a Becker and Hickl Time-Correlated single photon counting module.

The data shows that the feature patterned with 5 mW is significantly brighter than the other features (see image below):

Example #2

A monomer mixture of the following composition was prepared: 1mL hydroxyethyl methacrylate 2.6 mL Trimethlol propane trimethacrylate and 36 mg azobisisobutyronitrile. They were then sonicated for 5 minutes. Nitrogen was bubbled through the sample for 5 minutes. A bioptecs FSC2 chamber was purged with argon and then filled with the nitrogen flushed monomer mixture, with coverslip functionalized with trimethoxysilyl propyl methacrylate as described in example #2. The chamber was mounted on modified Prior scientific Proscan stage attached to a Nikon microscope. Laser exicitation was obtained from a Spectra-Physics Tsunami mode-locked Ti:sapphire laser (742nm), which went through a Conoptics shutter and was later doubled to 371nm. This then was focused through a Nikon 0.30 NA 10x objective onto the sample. Laser power was set to 250 microwatts going into the laser with approximately half of that power at the sample. The photopolymer was patterned using in-house software, designed to control the stage and shutter. The following patterns were made:

Position	Power into laser (μW)	Exposure time (ms)	Number of features	Feature spacing (μm)	Focus vs. cover glass (μm)
1	500	500	5x5	1000	250 below
2	250	1000	5x5	1000	250 below
3	250	1000	5x5	1000	250 above
4	250	250	10x10	250	250 above
5	250	250	10x10	500	250 above
6	250	250	20x20	250	250 above

After patterning the chamber was rinsed several times with dimethyl formamide (DMF) to remove unpolymerized monomer. The hydroxyl groups of the polymer were coupled to a NVOC protected glycine (NVOC-gly). This was prepared from the amino acid and NVOC-acid chloride using schotten bauman procedure. The NVOC-Gly was activated with the coupling agent O-Benzotriazole-N,N,N',N'-tetramthyl-uronium-hexafluoro-phosphate (HBTU) using the following procedure: 21 mg NVOC, 24 mg HBTU and 1 mL DMF were mixed and allowed to react for 30 seconds and 12.4 μL Diisopropylethylamine (DIPEA) was added, this mixture was allowed to react for 3 min before adding to the chamber. This mixture was allowed to react in the chamber for 30 minutes without mixing and then another 30min with recirculation.

Any unreacted sites were acylated with acetic anhydride: a solution of 5 mL DMF, 146 μL DIPEA, 100 μL acetic anhydride was prepared. The chamber was flushed with DMF and then filled with this solution and allowed to react for 30 minutes. The chamber was then flushed with DMF and then filled with dioxane.

Features were deprotected using the same apparatus used to pattern the photopolymer. The laser was set to the same wavelength and the power was set to 500μW. For the larger patterns (1, 3, and 4) by manually finding the feature to be deprotected using the microscope and opening the shutter for ~ 30 seconds to expose the feature. The smaller

patterns (5and 6) were deprotected by scanning adjacent features with the laser beam in a series of parallel lines forming squares. Position #5 was scanned with six 700 μm squares with 700 μm spacing between squares and each square was composed of 30 scan lines scanned at 1% of the maximum scan rate and 1 mW power into the microscope. Position #6 was scanned with 312 μm squares with 448 μm spacing with 20 scan lines at 1% of the maximum scan rate and with 1 mW power into the microscope.

After scanning the dioxane was drained and the chamber was flushed with DMF. The chamber was then filled with 10 mg/mL fluorescein isothiocyanate (FITC). This was allowed to react for 15 minutes. The chamber was flushed with DMF and sat overnight filled with DMF. The chamber was then flushed with fresh DMF and imaged.

Imaging was preformed using the same scanning laser apparatus and laser configuration as used for the patterning and deprotection of the polymer-NVOC-GLY. The laser was set to 8 μW input power into the microscope. Emission from the FITC was collected by an APD detector. Imaging was done with in-house software using a Becker & Hickl GmbH SPC-830 high performance photon counting board. All images were 64x64 pixils with the stage moving at 10% of maximum scan rate.

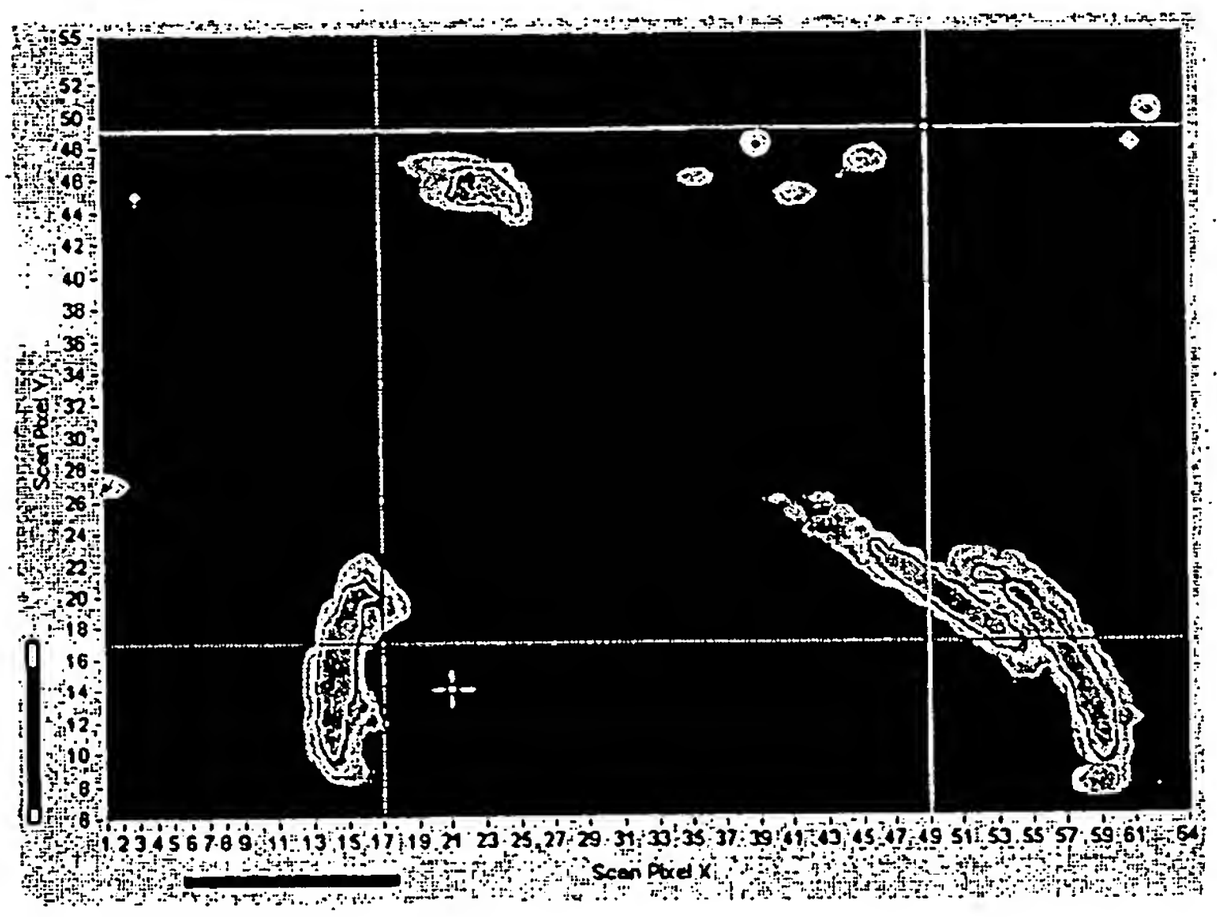


Image 1: Position 1 Shows the ordered spacing of the features (dark spots are unpatterned bright spots and lines have FITC. Note that the polymer is long hair like structures, some of which have fallen over.

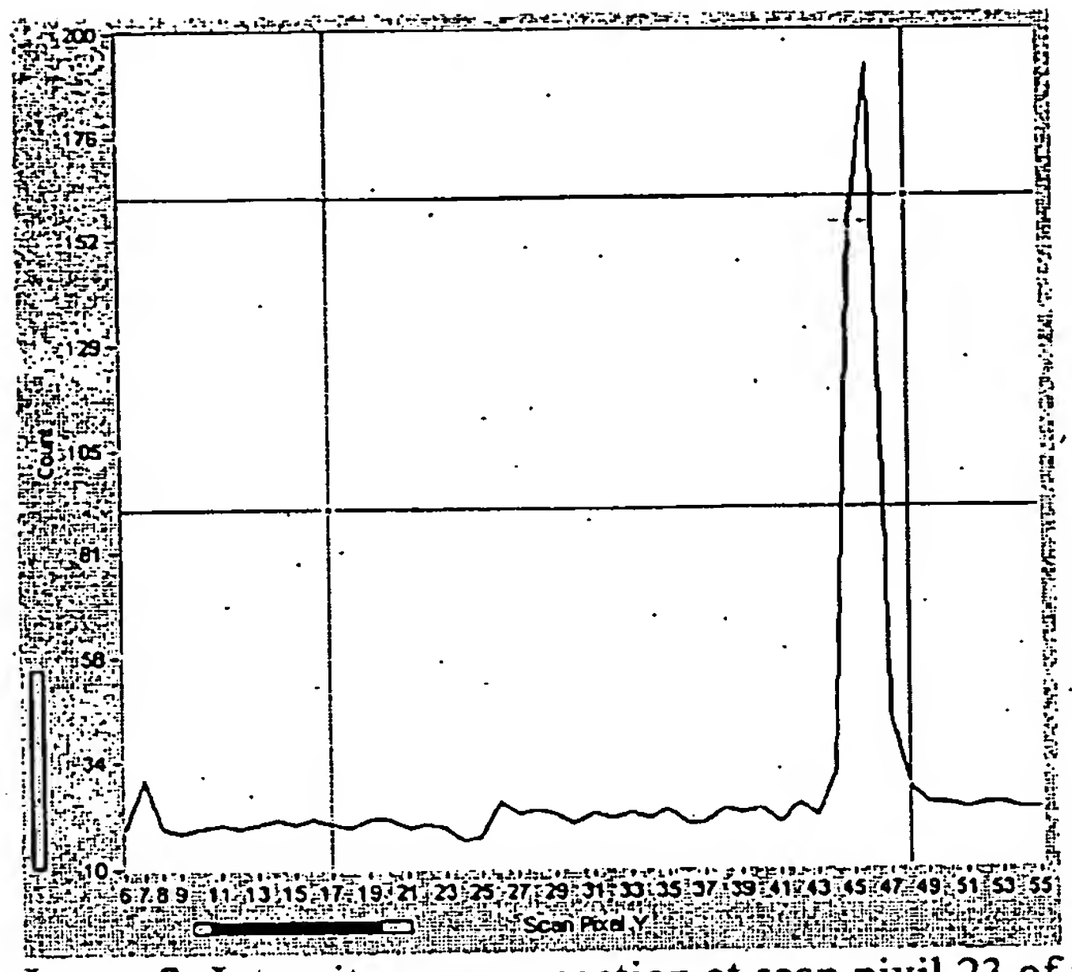


Image 2: Intensity x cross-section at scan pixil 23 of position 1. Note that the dip at y=24 corresponds to a unpatterned feature and the peak at y = 46 corresponds to a patterned feature. The image scan spacing was 50 μm so the two features are ~1mm apart which corresponds to the distance between features in pattern 1. The contrast ratio is very high since the unpatterned feature is darker than the background.

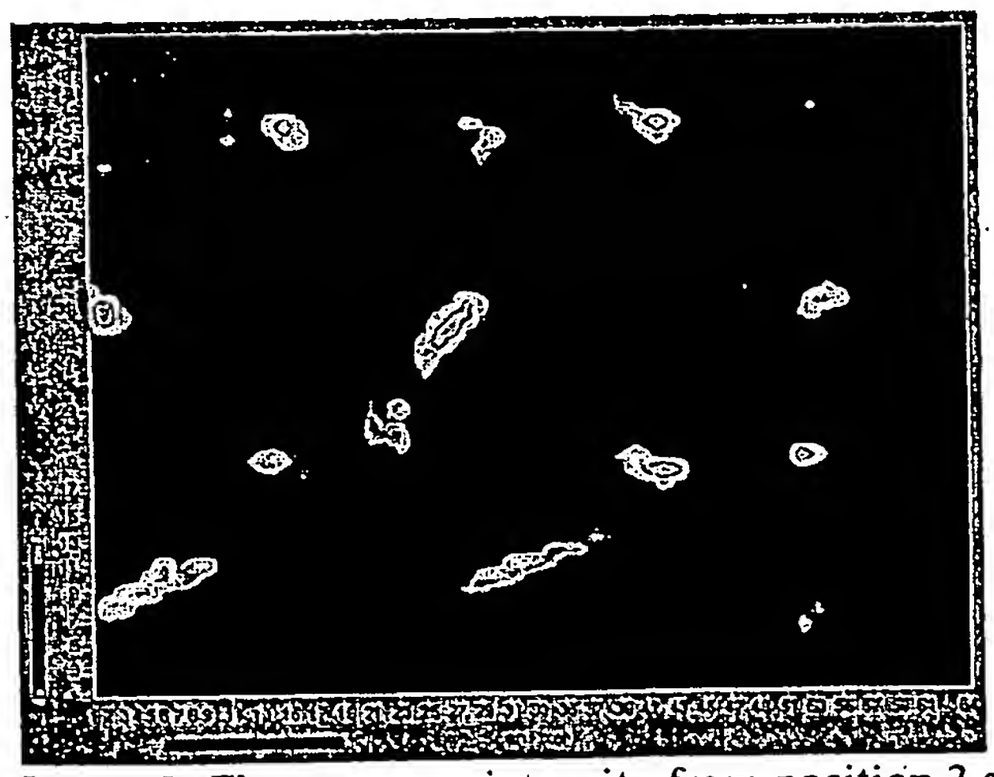


Image 3: Fluorescence intensity from position 3 shows alternating features of protected (dark) and deprotected (yellow, green, pink).

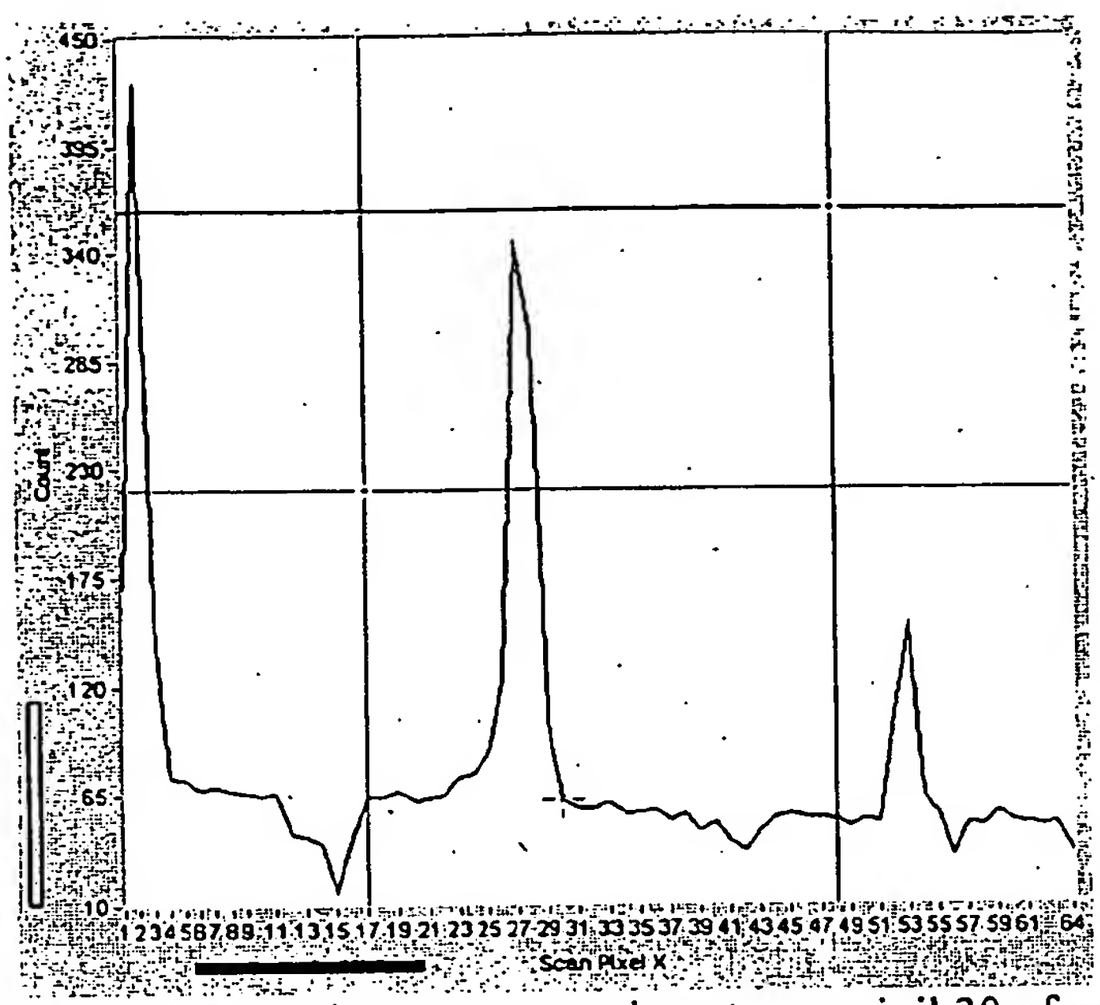


Image 4: Intensity x cross-section at scan pixil 30 of position 3. Note that the dip at x=15 corresponds to a unpatterned feature and the peak at x = 2 corresponds to a patterned feature. The image scan spacing was 75 μm so the two features are ~1mm apart which corresponds to the distance between features in pattern 1. The contrast ratio is very high since the unpatterned feature is darker than the background.

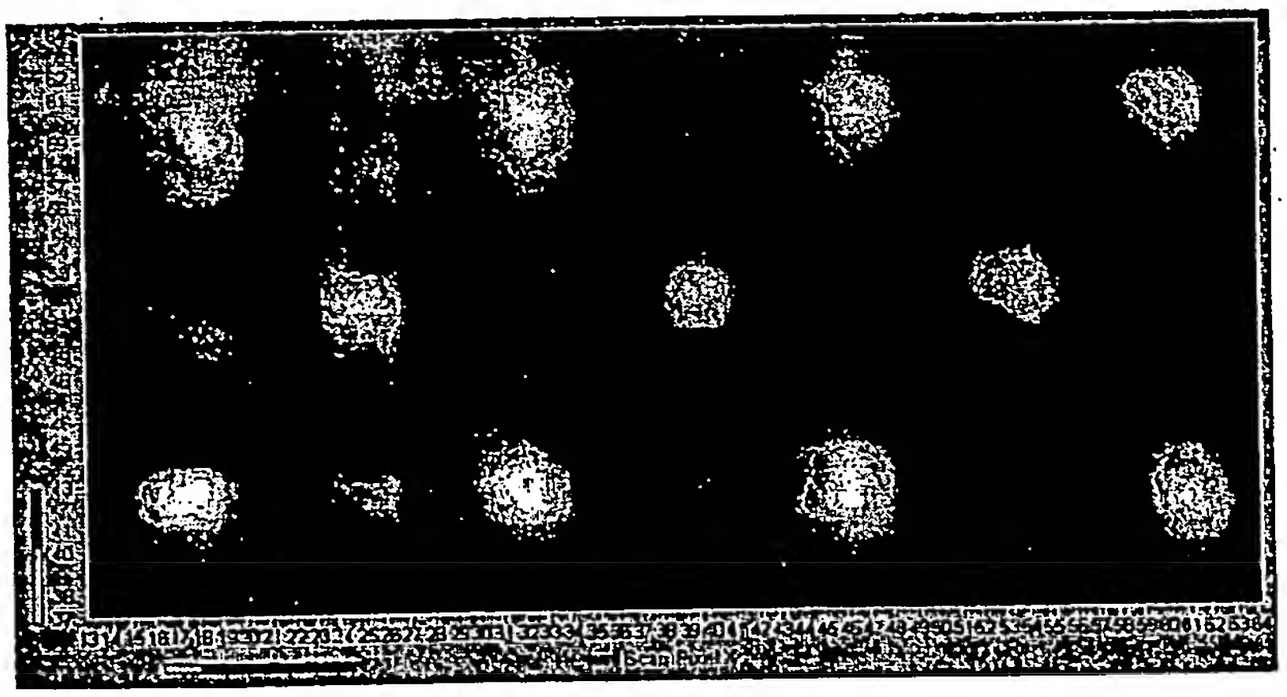


Image 5: Fluorescence intensity from position 4 showing the alternation of protected and deprotected features.

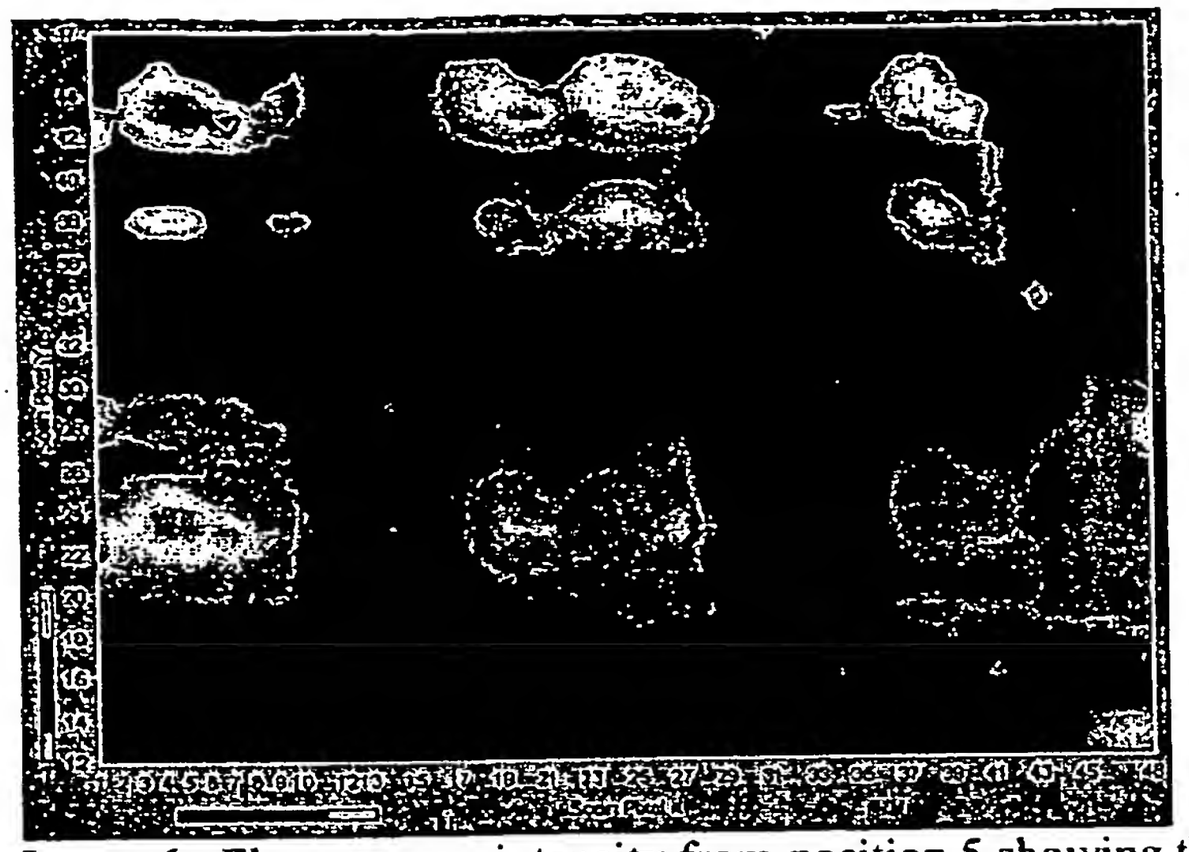


Image 6: Fluorescence intensity from position 5 showing the box like deprotection pattern.

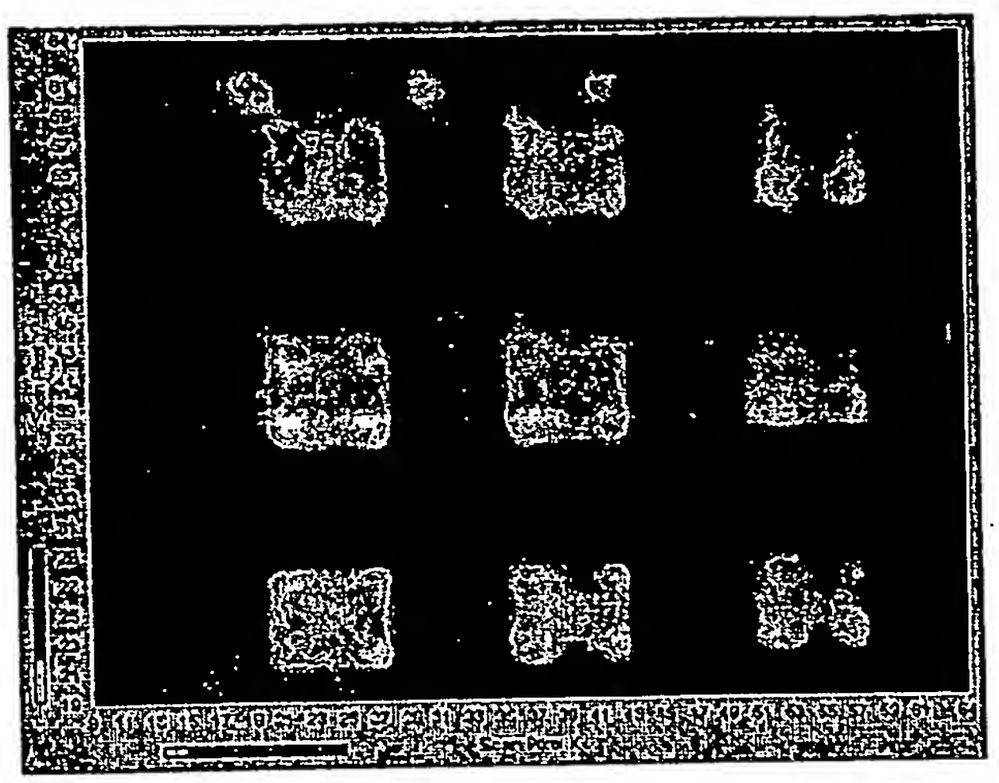


Image 7: Fluorescence intensity from position 6 showing the box like deprotection pattern.

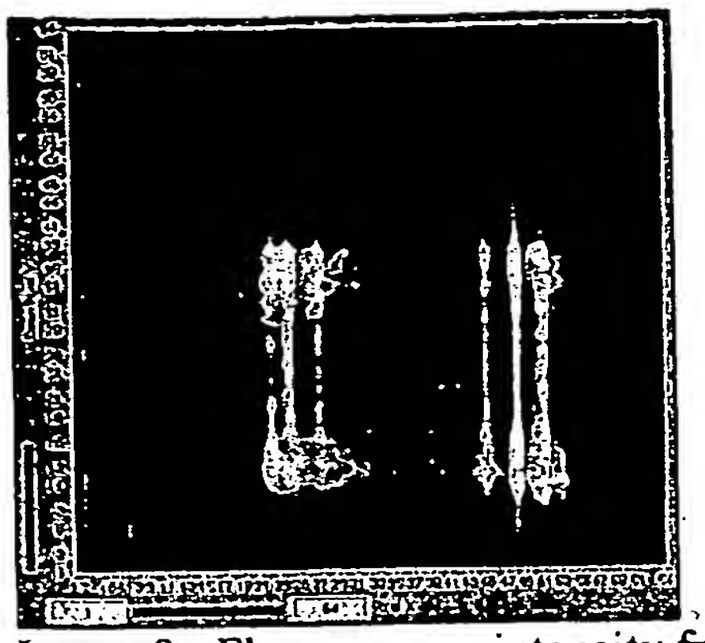
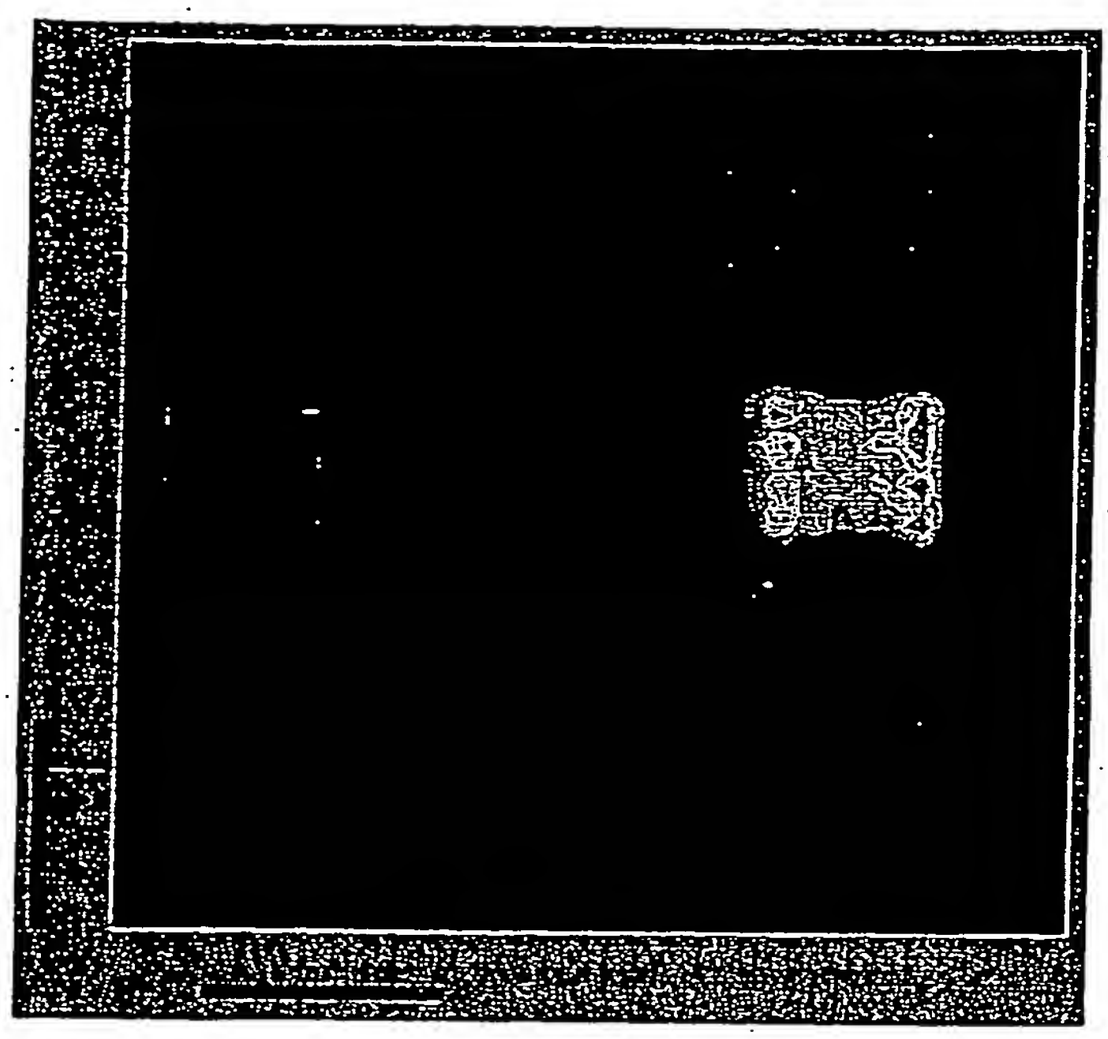


Image 8: Fluorescence intensity from position 6 showing the box like deprotection pattern of small features note that the features are 4 pixils in diameter and 25 pixil spacing, with a 10 μM scan spacing that is 40 μM and 250μM spacing as expected.



Claims:

1. A general method to create spatially defined complex polymer structures through sequential deprotection and addition of polymers/monomers to photopolymer structures.
2. A way of generating polymer arrays using photolabile groups with acrylate and methacrylate monomers and AIBN and its derivatives as a photoinitiator.
3. A method of enhancing the signal from microarrays by constructing the microarrays on patterned photopolymer arrays.
4. A method of generating three dimensional structures functionalized with a plurality of spatially defined functional groups.

Materials: 02/20/2004

Seed ✓ ppt x 3 300 µL in a stock

ResShape SL 5510 SLA System from Vantico

Propylene Carbonate Rinse 10 min on mixer @ 100 rpm

Isopropyl Alcohol Rinse Dry w/ N_2

Ti:Sapphire laser 10 in FWHM 80 mHz 730nm / 2

10x 0.7 NA objective

#	Amount	Scan Speed	Line Spacing	Power	S.A.	Obj
1	'ASU'	1%	40µm	1µW	5.5 x 5.5mm²	10x w/ 2x mode
2		1, 3%, 100%	40µm	100µW	5.5 x 5.5mm²	10x w/ 2x mode
3		1, 2, 3, 4%	50µm	100µW	4 @ 1mm	
4	'ASU'	1%	40µm	100µW	6x6 mm	TOOK A LONG TIME
5		Repeated #4				
6	2# (Small 'ASU')	1%	9µm	10µW	500µm x 500µm	All Look Great
— 16 Bars	500 x 500µm	1%	50µm	10µW	(4500µm)²	
— 16 Bars	100x100µm	1%	10µm	10µW	(900µm)²	

* IDEA ① Use Poly acrylamide attach PEPTIDES
② or Epoxy / methacrylate & decane ! attach PEPTIDES

Make an array of PADS Leave half of surface with Serums

Continued on Page

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PROJECT 2 hv patterning of SSTO polymer Notebook No. _____

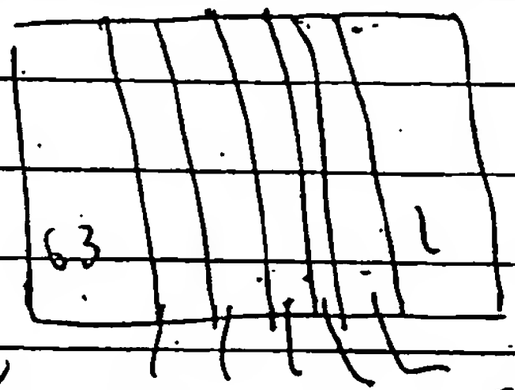
Continued From Page _____

Purpose: #1 to determine the Threshold for photopolymerization w/ 2hv excitation @ 730nm

↳ 11nm FWHM

LASER 730nm output after Beamsplitter 1 before Shutter = 650

output from objective 250mW 0.8mW



'Grayscale M'

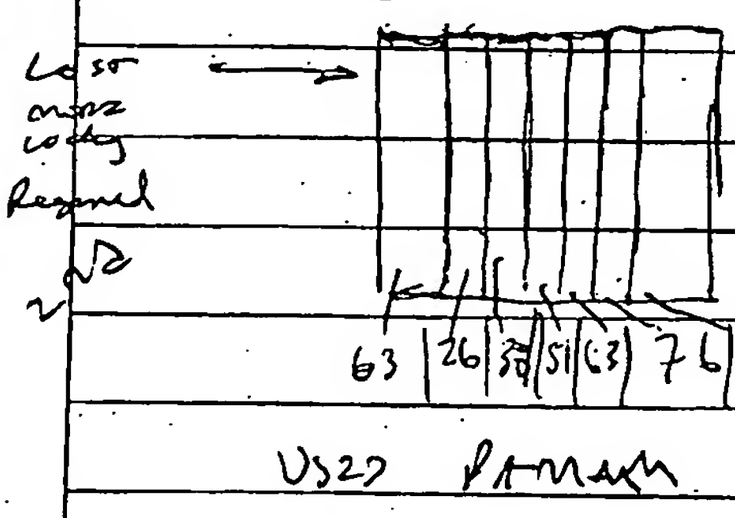
400nm x 400nm 8 bit B&W.

x step = 2 nm

y step = 6 nm

Scan Rate % 26 38 51 63 76

This would but the pattern came off when I washed/wiped it may have been focused above the surface.



Laser at 240mW 10 x objective

1900nm x 650nm

10nm x steps

10nm y steps.

USED PATTERN PREPARED 1/13/04 as a substrate

There was some polymerization only Area where there is significant polymerized vs between areas (where the Sinner is changing Scan Speeds need to significantly slow Scan Speed There are a variable Range where I get more Loss

Continued on Page

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PROJECT Attempted of SS10 photo initiated polymer Notebook No. _____

Continued From Page _____

Purpose See if it is possible to couple a Diamine to irradiated Epoxy Resins on the SS10 polymer

23 mL Bisamino Propoxy Butane + 30 mL DMF

Spincoated 6 x 9 (250 nm)^2 Rows w/200 nm Spacing : 9 nm Line Spacing @ 2 mW w/ 40x objective 50% SS

Rinsed w/ propylene Carbonate for ~3 min

Rinsed w/ IPA & Dried w/ N_2

Right to Diamine Solution @ 4:15 pm

Scanned same but w/ 100 nm intensity @ 100% SS

Still looks like TOO much

Try 10 mW for 1st 3e Coats & 1 mW for the Next 3 threshold

Switched to 1 mW for the Next

@ 10 mW Made structures like this

but I see anything @ 1 mW

need to either Spincoat polymer or Use too photo. concentrated

- Sample left in Diamine + H2O Rinsed off Glass Results a Small piece & Washed w/ more & Placed in Light -- Very Soft until Cured

- May want to Come while reacting w/ Diamine

Expose Tried two samples (1 w/ & 1 w/o Diamine) to 100 mJ/cm^2 Dosage 405 nm then Rinsed 6x w/ DMF

Dried 50 w/ more

Diamine is unbelievably fluorescent - not very if at all fluorescent

Epoxy Has Good Resistance to Non-polar Solvents (Hexane DCM, THF, Acetone - to

So this may be a possibility

Continued on Page

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Not DME

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PROJECT ~~SAW int = SAW set at objective int.~~

Notebook No. _____ 67

Continued From Page _____

Start ~1 SAW input ~ 1nW output from 400. 1 J/cm² @ 88% SR

Start high ! Go low Causes maximum of Black Growth

1st Scan 21

	0.25 J/cm²	0.125 J/cm²	0.062 J/cm²	0.032 J/cm²
	#5	#6	#7	#8
	4000, 0	4000, 3000	4000, 6000	4000, 9000

SAW Focusing Microscope

	9 J/cm²	3.5 J/cm²	1 J/cm²	0.5 J/cm²
	#1	#2	#3	#4
	0, 0	0, 3000	0, 6000	0, 9000

1st scan	300 × 300	w/ 150 spacing	20 Scan Levels	3×3	5R=10%
2nd	Same			5R=25%	14 μm Sp. 3.5 J/cm²
3rd	Same			90%	14 μm Spacing 1 J/cm²
4th	"	"	25 nW input	90%	14 μm Spacing 0.5 J/cm²
5	300 × 300	Same	1.25 nW input	90%	0.25 J/cm²
6			625 nW	90%	0.125 J/cm²
7			313 nW	90%	0.0625
8	200 × 250	5 lines	5 μm Spacing	15%	0.032
9	Same				

Divide by

* Move at Significant Scatter off top Layer !

~~Solvent~~

added dye 10 mg/ml Ground + Sand Blast for 20 min

* Focusing is very difficult Focus Bottom of Slide

Went up ~100-180 microns

By AFM on the Slide

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Continued on Page

2 250 50 44

1 100 4

Looking at 545 RF 35 w/ 500nm pass input

Bkt is 700 Fluorescent Imaged (20 - 1600)^2 15×15 p.

Lots of fragments (faintly Angel Cognoli) on Surface

Images in Neuron

Pos-Foo-V	X=12	Between Future Y=80	
	Image intensity	Background intensity	Comments
1 Image (3)	~~100-550~~ 100-250	103 90 ~~111-105~~ ~~120-Blue~~	No visible fibers
2	381	134	
3	250	134	
4	~~128~~	129	
5	155	129	
6	155	129	
7	155	119	
8	155	119	
9 (900, 7000) Background	155	119	

Over-all Observations: Very Splotchy

I don't think this instrument program is working well as it was when first ~~gets~~ set up the B-12 cap & looks like it has the same topography of the others

Next Bobs is working with #1 looks the best how to make out any features after #3 ~~7~~ J/cm^2 might be perfect

Continued on Page

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Try Dry Mix Concentrated down in THF. For a Stock
of 5-09 426 µL Bis-Amino Propoxy Stock in 1mL THF
~180 mM
1.0 mW
First Screening w/ ~~0.1 mW~~ w/ 10x (2000 µm)² 25x25 Lines
1 µS exposure
Seems 0.5 mW Some
0.25 mW Some
0.1 mW Some
No

The Stamps were too High! They fell at a Reduction

Tried w/ 40x (2000 µm)² 20x20 Lines.
They don't stick to the glass very well
Very close to APTES Surface

Keep working w/ 10x ... can always (Hopefully) Scale Down
250 mW 10x 1 mS/pixel (4000 µm)² 20x20 Pixels
200 µm between Spots
Put on DC Applied gently until excess had dissolved
Put microscope lamp on it for 5 min while in the
fe

Photosensitive Seems to Require 550 W very well
125 mW w/ 90% SR! 10x is right at the threshold
only the edges photocured.
Spots vs. not interesting

Tried Making Some White "Ham"
1st wash @ 125 0.2 mW
Next wash @ 0.3 mW

	.2
	.3
	.5 4000/200
	.75
	1 mW

Continued on Page

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1000 µm x 400 µm 25 x 25 Lines

World with Crazy Though 80 mW Spray Work 75

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Imaging 'Hairs' from pg 71
w/ DAPI filters excite @ 365nm Got a 'Huge' fluorescent Signal. Probably from photo reactors.
Need to take an excitation & emission Spectrum from Polymer to figure out if I can find a dye to use with it / where it will have a small Stokes Shift so that it is bleaching white light.
Imaged w/ 40x 40NW. Features are 25μm in diameter & have a S/N of 80 Much higher 1160 = 5 N = 78 (Dark Counts) = 5
WHAT WOULD HAPPEN if you Shattered balls into these. 1160 / 13 = 9
You would have a SPR System?

Power dependency of emission

Input Power (μW)	APC	Input Power (μW)	APC
0	7.522	90	7.6 E6
2	7.8 E2	80	2.5 E6
3.4	8.7 E2	70	2.3 E6
5.4	9.8 E2	60	2.2 E6
8.6	1.1 E3	50	2 E6
11.2	1.2 E3	40	1.8 E6
16.6	1.4 E3	30	1.5 E6
23.4	1.7 E3	20	1.1 E6
39	2.3 E3	10	6.4 E5
		9.7	5.7 E5
		8.3	4.8 E5
		3.6	2.9 E5
		1.2	1.1 E5
		.8	7.2 E4
			1.5 E3

Not well correlated

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PROJECT 3/16/04

Notebook No. [illegible]
Continued From Page

[illegible]

Excite	Em			
365nm	375–615nm	450	3656	
400	453–650	475		
500	585	538	3322	
450	550–700	505	528	2871
380	381–570	441	538	1947

Excitation
Emission @ 800nm

Excite	Em
365	338
400	734
410	775
420	868
430	1008
440	[illegible]
450	1306
460	1474
470	[illegible]
480	1660
500	[illegible]
550	[illegible]

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PROJECT

Notebook No. _____ 75

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Emission	Corrected @ 550	Emission	Emission @ 500	
450	2423	450	3341	
475	2966	440	5039	
500	3567	430	2470	
425	2100	420	3055	
400	1751	410	5234	
365	1083	400	3437	500
350	515	365	2771	420
		350	1252	460

100nm Step Shift

Excite	Emission	I
350	450	1295
400	478	2913
425	500	2278
450	527	2177
475	549	1492
500	534	2330
365	447	2721
550		

Not Very Soft Scatter

515 may be the Rayleigh excitation wavelength. Need higher wavelength

00 = 3 @ 375nm
off scale @ 365nm

Absorbance

1

00.6

400 500 600

Continued on Page

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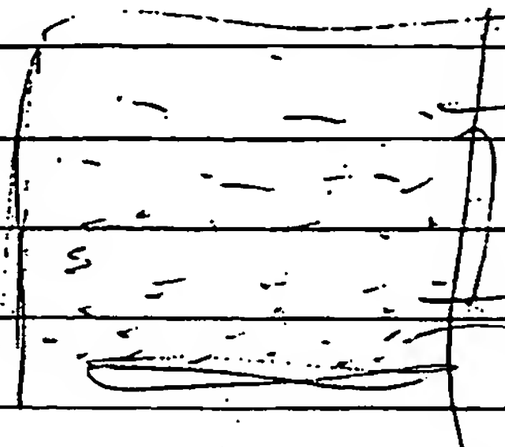
PROJECT Patterning holes out of SiO2 w/ HF Notebook No. _____

Continued From Page _____

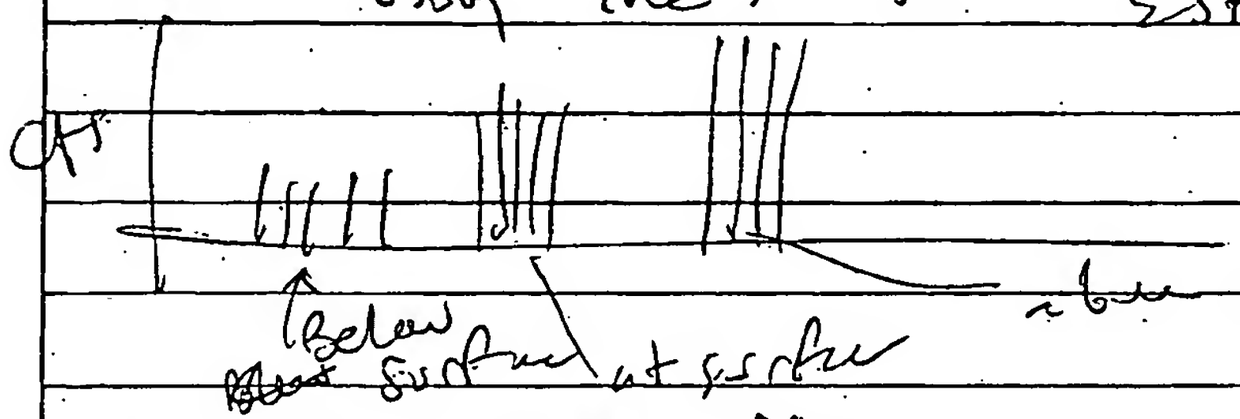
Using new objective 36 mm extension

APTES Silane

3000 x 3000 nm 30 pixels x 30 pixels Shutter speed 1 ms



Using new to establish



No 20 nm 40 nm

Brought in Sealer of acetonitrile

mixed for 6 sec then put onto a hybrid (or microscope)

for 5 min

Features are ~ 5 nm wide & 20 nm long.

Tried to make longer features by using thicker

coating placed 2 150 nm layer SiO2 on APTES Silane

& used a 2:2 CS to spread ~ 40 nm of polymer on

surface

30 nm pores (1000 nm x 1000 nm)^2 25 x 25 pairs LANS / pixel

40 nm Spacing

Worked well except features fell over ~ 80 nm long & 25 nm

Continued on Page

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Date

Craig Lamagni

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stand-up By curing in place stay low intensity
for a long time.

18 J/mm² for 2hr w/ SNL-500

from paper 31 Rather than high intensity

300 mW for 1 ms try (10mW for 30 ms)

if this doesn't work try

Pulsing twice; once to flatten & the times from the

cure

□ 3 ms

□ 300 ms

□ 30 ms

Pulsed Using mes Lens.

10 mW 10x objective (1000 nm)² / (10 pixels)²

300 ms (300 ms, 3 ms

3 (only one that worked, Hardly at SNL ones

that stands up.

Circle

Feature width ~ 25 μm in diameter

(melted w/ 100mW for 1 ms w/ 40x

worked well)

3 ms @ 10 mW (1000 nm)² / 10² } Both appeared to

3 ms @ 100 mW 3x (800 nm)² / 8² } work

Don't stand up as well

Tweaked w/ 40x: 40 μW too much power

Very long fibers ~2200 μm long 2 μm W.X

Limestone 400 x 400 nm / 30 x 30 @ 10 μW w/ 40x

~70 nm across ~200 nm long

This is how to know when the

Beam is 13x w/ 40x probably 10 μm x 150 μm is max accurate.

Continued on Page

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PROJECT _____ Notebook No. _____

Continued From Page _____

Now trg w/ 40x

w/ 10x $\frac{(8\,\text{Sec})(10\times10^{-6}\,\text{w})}{\frac{\pi}{4}(12\times10^{-4}\,\text{cm})^2} = 35.0\ \text{J/cm}^2$ VB $25.5\ \text{J/cm}^2$

meas 800 ms w/ 2 mW input w/ 40x

found so w/ 0.2 mW input

The MCS method of focusing works well: looks for the Maxima closest to the surface

Everything has collapsed!

EXAMPLE scans 1st scan to 2nd scan w/h VBH for Nervous Polym

BAW	40x	1 ms	1 ms	0	0
"	"	1 ms	10 ms	1000	500 x 500 nm / 10 y/10 pixels
		1 ms	100 ms	2000	0
		10 ms	10 ms	3000	0
		100 ms	100 ms	3000	3000
		100 ms 200 ms	0	2000 3000	2000 3000

Stretch the SSLO by Blowing Argon across the surface.

guessing that

only the 100 / 100 [illegible] ?

Still felt over [illegible] by our pattern Came over as [illegible]

Continued on Page

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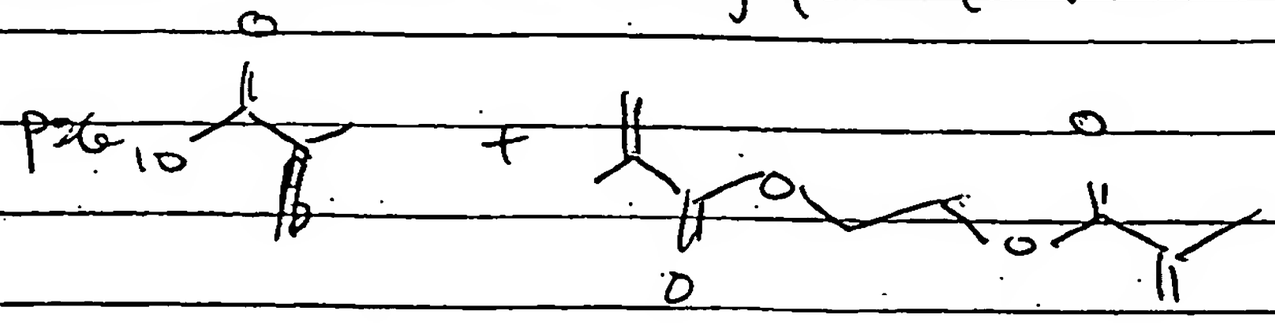
80.
PROJECT 1st Attempt at Methyl Acrylate polymers.

Notebook No. _____
Continued From Page _____



P26,10MA EGDM

1st Attempt	1st Attempt	2nd	3rd	4th	5th
P26,10MA	0.9mL	450mL	45mL		
EGDM	1.9mL	10mL	10mL	100mL	100mL
AZO IBN	11mg	0	2		10mg
Ethanol	1mL	0	6		50mL DMF
Benzophenone	0	6mg	28mg	50mg	
	Methyl	Methyl	Some film formed		worked after a while

NEED to THINK ABOUT kinetics... NEED TO REMOVE INHIBITORS!

NEED Amine Triethylamine @ 50mM

1st Initiator		1st	2nd						
2nd monomer									
		1	2	3	4	5	6	7	8
EGMA	50-98%	98	98	98	98				
AZO IBN	1%	1	0	0	0				
PB	1%	0	1	1	1				
TEA	.5-1%	0	0	1	0				
BAPB	.5-1%	0	0	0	1				
BTOA	0-48%	0	0	0	0				

amine initiator amine

Continued on Page

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removed inhibitor by running over Sigma-Aldrich
column.
test washed column w/ a tolt
for ferric & dissolved O2 prt
flushed w/ N_2
keept under N_2
polymerizing in Beaker

$\left(\frac{50 \text{ mmoles}}{L} BP\right)\left(\frac{182.2 \text{ mg}}{\text{mmole}}\right)\left(0.001 L\right) = 9.1 \text{ mg} \sim 1\%$

① 10 mg Azo B:C 1306 dtgo Nitrile + 100 µl Cyclens grade DMF & 900 µl MA
② 10 mg BP + 10 µl TEA + 1 mL MAA
100 µl ① + (10 mg Polystyrene pyrolidone + 100 µl DMF)
200 µl ② +

The samples w/ PVP worked significantly better than those without.

Made $\frac{20 \text{ mg}}{\text{mL}}$ Azo in DMF $\frac{60 \text{ mg}}{300 \mu l}$

made $\left(\frac{10 \text{ mg PVP}}{200 \mu l \text{ MMA}}\right)$

added 50 µl of Azo Solution
worked ok, not sure if it is any better than 7°C
Need to flushed even more.
Need controls & understand what is happening

Continued on Page

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PROJECT

Notebook No. _____

Continued From Page _____

	MAP	DMAP	PVP	BL	A20	TCA
1		X	X			
2		X	X		X	
3	X					
4	X				X	
5	X		X		X	
6	X	X		X		

#5 10mg PVP + 10ml of 20%
MAP + DMF + 180ml MeOH

Exposed 2 min (crept for 3 min w/ 10x

OBSERVATIONS:

Before modified off Excess #4 & #5
have cloudy material on top. The others
are clear

4, 5, 6 are the only ones that glistened
4 is white!
5 is clear but has lumps very near
6 is very small & clear.

∴ formula Number 5 seems to be a good
starting point.

Exposure test with formula #5 2.1mW w/ 10x

1 Sec Barely visible spec

10 Sec Has < 100 μm wire probable Choice to 20

30 Sec ~ 200 μm

40 Sec 1 mm wide

Good enough for Now

Continued on Page

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Craig L Maylon
Signed

4/21/04
Date

Trying adding DIAMINE 1%

① 10mg BVP + 2mg P2DA (1500g/mL) + 10mL DIAZO in DMF + 178mL MMA

② " 2mL Bisomer no polymer Before "

Patterned ①:②: #5 from before 2.1 mW 10x 30 sec

Worked w/ Acetone

all Spots lost the Same

Stained w/ 10mg DANSyl Cl + 500mL DMF + 10mL DIPEA

4 30pm left 1 of two #① w Stained one on edge

4 45pm

See bgnds, but Both TBZ #5 + P26 + DANSyl &

#5 + DANSyl are fluorescent #5 + P26 is

1/10 as fluorescent (excitation @ 365nm, 30% mW 40x)

This Maybe ok. Need to determine if the dye

is REACTING w/ surface or is simply being adsorbed.

Test By Acetone the surface & Staining w/ dye

+P26 P26 +P26 P26

0 0 0 0 Use #5 + P26 formula

w/o 4 acetone only rinse

Exposed to 2.2 mW 10x for 3 sec

+ P26 Hx at RT 2J - P26

Acetone Anhydrous Soln: 0.9mL DMF + 100mL AA + 100 μL DIPEA

Dissolves polymer ('uncured') after 5 minutes

cured polymer - allowed to stay overnight but didn't dissolve.

& it swells in DMF - Hard to rinse dye.

Needs to cure under lamp for ~ 30 min Continued on Page

To continue.

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PROJECT _____ Notebook No. _____

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Rinse GMA w/ Column 18" x 0.5"

1st run 10 mL 9mF

2nd 10 mL EtOH

~~Final~~ N. Blow dry Stock

Run GMA, do not polymerize w/ 1% H2O

Run A Screening Form (re run)

	%w v Inj				Result
	A20	MMA	GMA	PEGDMA	
1	X	X			Works turned white?
2	X			X	Clear works (30 sec)
3	X	~~80~~ 60		~~40~~	worked well fast!
4	X	~~80~~		20	
5	X		~~80~~	~~40~~	Slow (60 sec) Clear
6	X		X		Nothing
7	X	20	5	75	
8	X	~~40~~ 30	20	30	very slow (almost)
9	X	35	5	60	turned the last. Still very slow
10	X	75	5	20	Turned white @ 30 sec

Adding 1/2 BAPO in DMF to #7 w/ 10 mM had turned white Get pro? i.e. Must was still un-reacted? probably dissolved in DMF & reacted w/ plastic

Cure other half for 15 min & repeat ✓

reacted #9 ~ | Dimm for 15 min, no apparent change in physical properties

Added w/ DMSO (#8 & #9) for 15 min

Continued on Page

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~~2x w/ DMF~~

~~Very thin~~ Very thin resist & #8 is NOT.

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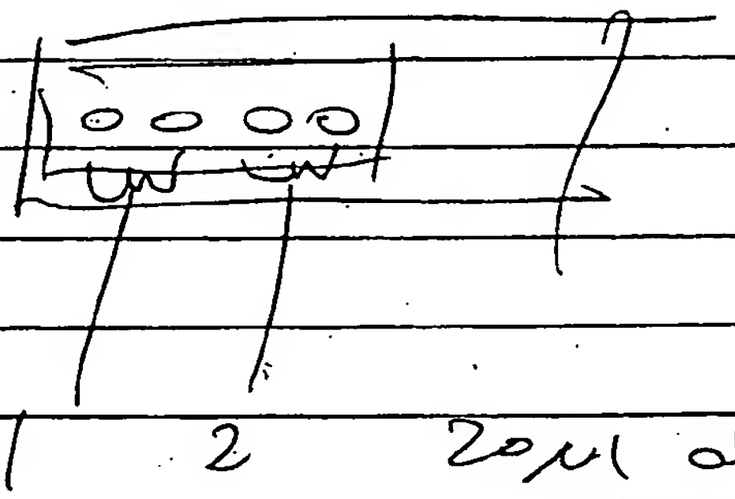
This is good #9 looks like a good formula, may want to
Label GMA to triple check that the inhibitor is removed.
This needs a way to attach to the oligo. I.e. needs
a way to confirm

Make more of #9 using GMA that has been
stored w/ Aldrich beads for 1 hr

Book like had the same issue
try w/o MMA

A20 22 100µL	GMA 100µL	PEG DA 800µL
100µL	50µL	850µL

Oops ended up adding 2% A20



1 2 20µL of each.

Has become fluorescent not A&T2s
PEG-DMA Yay became fluorescent! Probably from both
Go see Prof with PEG-DMA stand in. Empathy Mixed together
Dried 10% BAPO 1253pm

Try 50mL A20 + 100mL GMA + 850mL MMA

Continued on Page

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PROJECT _____ Notebook No. _____

Continued From Page _____

- Purified more PEGMA over Sigma Resin
- Recrystallized AIBN is BAD to Nitride from MeOH @ RT (AIBN

Indo ① 50mL AIBN + 800mL PEGMA + 50mL GMA

② " + 50mL GMA + 400mL PEGMA + 400mL MAA

50mL AIBN + 800mL PEGMA + 50mL GMA + 50mL BAPB

- Cannot go 60 Sec @ 2.5mW
- Needed both w/ Darkfield both were stored @ 2 more Hours #1
- This makes a soft polymer, probably the Bond is type cause the reason -
- The MAA seems to make it more crosslinked more rigid
- May be that the Uv is too much light getting thru polymers.

Try less light & blanky on some MAA

Use 200mW for 60 Sec w/

1% AIBN + 1% (BAPB + GMA) + X

X = 10 - 100% MAA : 0 - 70% GMA

Continued on Page _____

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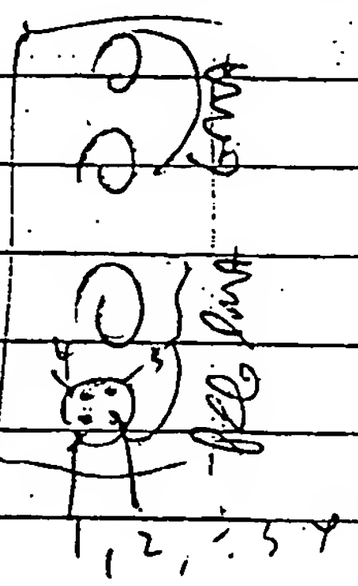
PROJECT ~~ArBot~~ polymerized

Notebook No. _____

Continued From Page _____

Ran 10 ml of ~~Ethyl~~ GMA through Aldrich Basic Alumina column (0.5" x 8")
degassed w/ fresh Argon for 20 min GMA
Added 1% AIBN (recrystalized from MeOH) Solution
Bubbled N_2 through for 2 min
placed on cold slide (20mL) w/ Cold Nitrogen IN and
out of N_2 flowing through. Oven Heated @ 85°C
11:45 am 4/6/04
12:45 pm Both samples cured into clear Hard polymers
The PEG doesn't stick to the glass very
well can't cut the GMA off the glass
All three are like Latex condoms
Lasers

Laser @ 365 nm 2 mW unit intensity
Warmed Sample to 50°C over 20 min & purged w/ N_2 for
twenty minutes Laser focused to 500 - 1000 μm



1 = 30 sec @ 2 mW
2 = 30 sec @ 200 mW
3 = 300 ms @ 200 mW
4 = 2 ml @ 2 mW

Continued on Page

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None of the light irrigated Polymercaptans Today
worked very well. The GMA stuck to the Glass like
Crazy. The PSH doesn't appear to stick very much
in DMF or water.

together they might work. Try various combinations
in afternoon

PSH wt%	GMA wt%	
100	0	Some Bonds stuck to glass
0	100	Stuck to Glass amazingly well
50	50	
99	1	Sprayed w N_2 for 1 min
90	10	Then spotted on plate in oven @ 80
75	25	forgot w/ N_2

finished in 15 min.

None stuck

All are clear at 1st set in BAPB (mist) 620 in 4/6/04

only Takes 10 min first mixed for Spray
The 90/10 cracked overnight, may not have anything to do
w/ formula

Looked

The set treated w/ DI Water is very fluorescent
even after washing 2x w/ Acetone ~ 380 w/ DMF
The set NOT treated w/ DI Water is not fluorescent
Looks!

Continued on Page

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PROJECT Trimethoxyketo formulations. Notebook No. _____ Continued From Page _____

w/ 12 ABN

	TMA	PEGDMA	GMA	
A	100	0	0	TMA 378.4 mg/sam
B	90	0	10	PEGDMA 178 mg/sam
C	45	45	10	GMA 114 mg/sam
D	99	0	1	3.5

A & D are very brittle, they crumble

B & C are much better

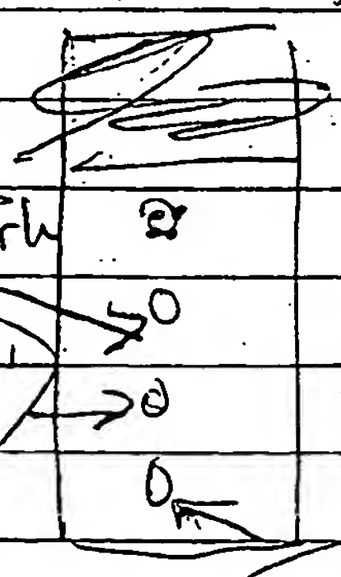
B + 10% Rhine in DME 220 psi

E 75

f

Degassed 4 2ml shots of B in oven (off) for 30 min

@ 350nm filter for 5 min (Dosing) The plate in slide 4 2ml shots for 5 min put in petri dish on Foil w/ Parafilm Cured w/ black cloth 30 min desired



1 sec @ 3 mW cm⁻² both worked

0 — 1 sec @ 300 mW Did not work

0 — 300 mW 30 sec Worked

0 — 75% 55 @ 300 mW (500×500 mm²) Did not

300 mW Did not work 50mm dear

* appears that you need a lot of light!

Continued on Page

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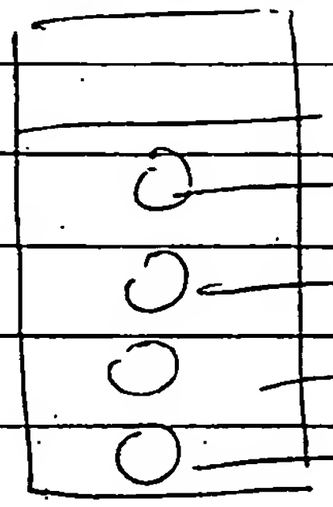
Took a Methanolic Sol. de put some of the slurry on it
Scanned on (1mm)^2 1000 lines @ 3 mW
all @ 32 mW



@ focus

Made new B 900 mL TMA + 100 mL EMA + 6 mg AIBN
Purged ~1 hr for 5 min Blew N2 through and placed slide in oven in same petri dish.

04/13/04 TMA 2-ammonium AIBN
HCl

wt % Soluble	50	50	12 mg
	90	10	12
	95	5	
	99	1	

04/14/04 Scanned @ 100 370nm
3 mW 99% Sc Rate
(500 nm)/10 w/ 600gm Spacers
Washed with ethanol/Acetone w/ Ethos worked OK

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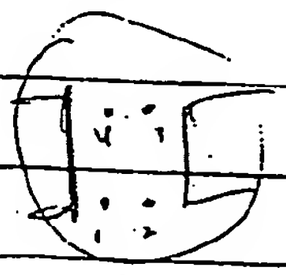
PROJECT Patterning dye on fluorescent microspheres

Notebook No. _____

Continued From Page _____

pulled 1 ml chamber Hela chamber w/ Argon
on shaker placed by Sarika Ring protect of methanol 5
900 ml PMA + 100 ml 60% A + 10 mg ABN
Bubbled N_2 through for 15 min



1. 250/50 500 nm spacing 100 nW ~~of 40 x 99%~~
2. 250/25 Same
3. 50/10 Same
4. 500/250 washed Best Same
500 nm spacing

washed w/ ether

only See Separation of beads on #4

@ 102 BTPB cover put for 15 min
~~Beads~~ Beads Came off Glass. Should have
Cured 1st (warmed to 50°C Blew N_2 through)

Mask Dye 3X

14 mg MMA 10 ml DPA + 500 ml DMF

Sonicate ~ 5 to 10 min

Cured 30 DMF

Filled w/ Dioxane

Separated 2 layers on petri #4

5 mW → 1 mW 100 SR 10 times

10 mg Methyl Methacrylate + 500 ml DMF 15 min

total ~20x by DMF to get Dye off (Continued on Page

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PROJECT

Notebook No. [illegible] 93

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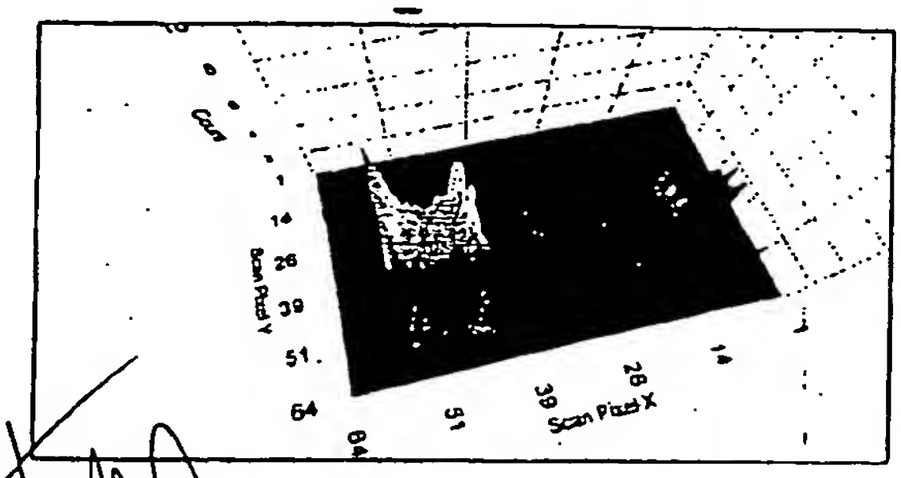
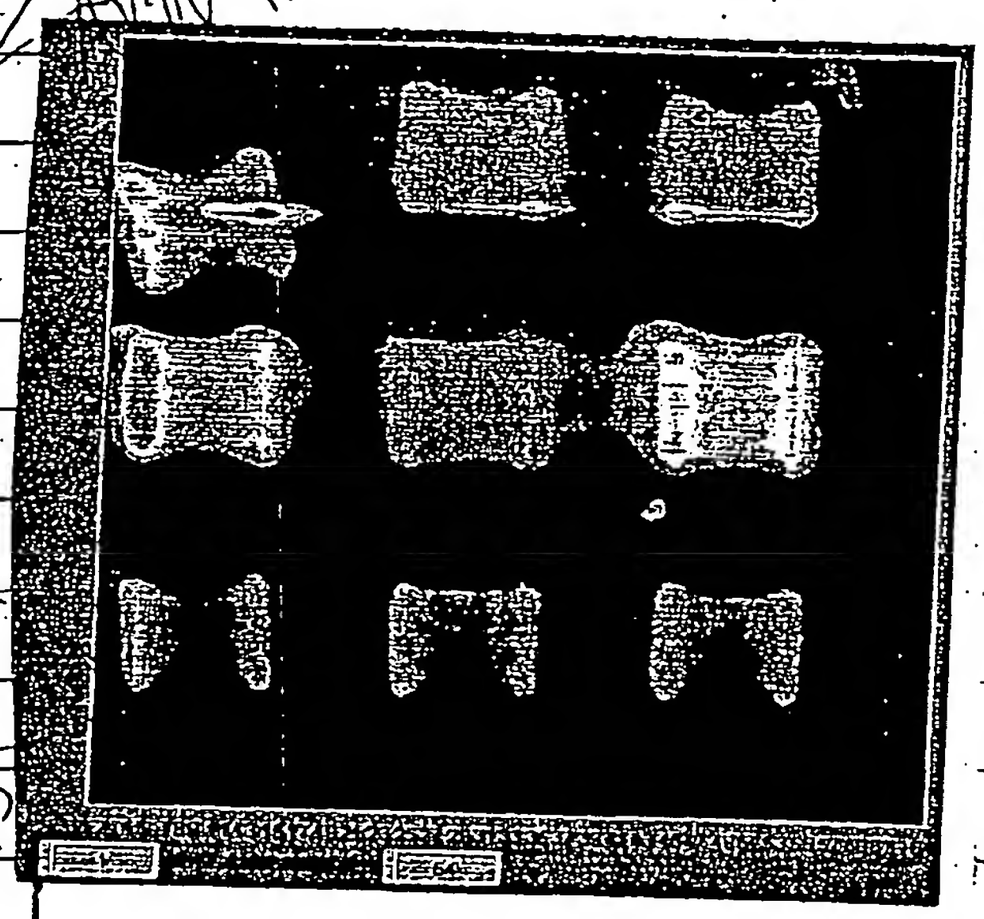
37 DAM 60X no fluorescence.



The TWO positions reported on were fluorescent that other positions. Through Several others are non fluorescent (thick scatter) I am not fluorescent (no exposure protection.



Reacted 4-15-04 after overnight in DMF 4mW 60s no pre Hole

There is a difference Between the 3mW & 4mW.

Why more Spacing? use 3mW 500/250 w/ 750nm Spacing?

AA Lap after MDC protein?

Why don't all of the features adhere to the glass?

Using 40x I can see that there are Reflectors in the holes as a result of the Scan Spacing. They appear to BE ~10nm Long w/ 10nm Spaces. It is further interesting to note that features 1 & 2 are fluorescent or if the dye or protein group was/was not able to penetrate the holes.

Continued on Page 97

(also was Read and Understood By Craig Magee I was)

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PROJECT 2nd Photo exposure pattern dry Notebook No. _____ Continued From Page _____

Two attempts:

(1) repeat but lap w/ AA

(2) Make more Hydrophilic polymer (increase from PEG w/ PVA

Try both

TMA / PEG / GMA 45/45/10

450nL / 450nL / 45nL 11mg AIBN

~~DISSOLVED~~ 15 min (BUBBLED N2)

Used mes TO FIND SOL.

(1) pattern 500 lps w/ 1mm spaces @100 mW 50% SR

(2) wash excess w/ ether

(3) Blow N2 through & Heat to 50°C for 15 min

(4) CMABz

(5) CO & BAPO in DMF 30 min

(6) NVoc 30 min

(7) AA 15 min

(8) Repeat Some pattern factors w/ 3 min

(9) Develop 15 min

(10) Rinse many times w/ DMF

1,4-Bis(3-oxiranylpropoxy)butane

Continued on Page

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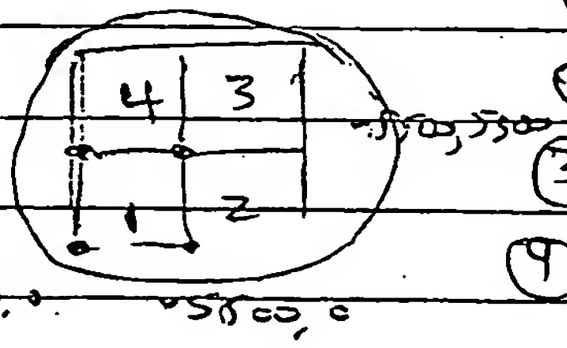
Craig Comaya Signed 4/21/04 Date

① The rest of getting 60 counts ~ 6 times what it was before
not sure if this is from remove pre-bake or that the
PEB is fluorescent.



① 500 / 25 w/ 1000mm Spacing 792 SR
② " " " 66% SR
③ " " " 33% SR
④ " " " 17% SR

#3 is more fluorescent (2x) than ① & ② DUV beam & can
Beam Shape

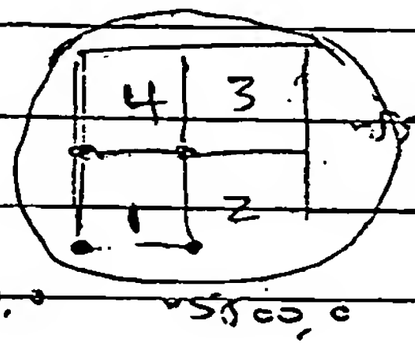
② - wafers 5x flushing Back & forth w/ PEB
w/ ETHAZ
Blow dry w/ N_2
Solvent Chamber in Spin Bowl
③ Heated to 55°C for 15 min while blow
N_2 through bowl

30mm w
Hole @ 200
370 nm

Page

4/04
Date

(1) The nes of getting 60 counts ~ 6 hrs what it was Before not sure if this is from reseeds pre-hybe or that the Pb is fluorescent.



① 500/25 w/ 1000nm spacing 792 SR
② " " ~ 4 660 SR
③ " " 4 332 SR
④ " " " 172 SR

#3 is more fluorescent (2x) than ① + ② Dehybe & can Scan slower

② Wash 5x flushing Back & forth w/ PBST w/ ETAZ
Bleach w/ N_2
Seal Chamber in vacuum oven
③ Heated to 55°C for 15 min while blowing w/ Through oven
Rehyb 5x flushing on + out
④ ~~Reseal~~ Seal w/ MHC + 10µl DAPI + 5 total ONF 30nm w/ orbital table @ 200 rpm
Imaged →

10x objective 992 SR 1000nm input power @ 37% w/ features on ~9 cuts

⑤ CAP: 146/5 30 µL DAPI + 20µl AA + 1mL ONF 15 min on orbital table @ 200 rpm

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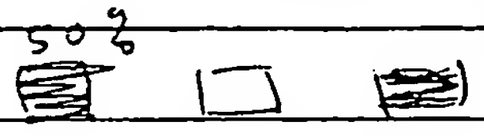
(7) Protect picture #4



10 lines for detection

w/ 10 x @ 2 mW each

(N 10mW Hz80y/ONF

Rad 30 w/ ONF

(8) Long shaft & Small dia + Small ONF
Place on table @ 200 RPM

Current will be off of Lathe. A larger
bit might make better w/ this system

Continued on Page

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	4/16/04	Craig Cmeyll	4/21/04

- I have been able to make smooth polymer features w/ the 5510 polymer as small as 2 mm. I have also made small features w/ my own methacrylate/AIBN formulations but I haven't quantified their sizes yet.

- I have imaged long hairs of 5510 photopolymer w/ aspect ratios ~ 100 with a confocal microscope. They appear to act as optical fiber sources. I see a huge fluorescence when imaging at the glass polymer interface.

- I have successfully protected and deprotected my methacrylate resin functionalized w/ Alanine. The protection was w/ NVOC & deprotection w/ the 355nm laser. Hydrolysis using basic chloroform. This should provide significant improvements in my contrast ratio vs. working on functionalized glass. Further I hope the surface chemistry will be better vs. glass so I will be able to synthesize long peptides w/ high yields using the NVOC protection deprotection scheme

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PROJECT Screening Formulations

Notebook No. _____
Continued From Page _____

Purpose: to screen formulations against the following criteria

① Rate of release of Absorbed dye (Rate of wash)

② yield of MMP protected cotton

Methodology: prepare samples, Spot onto RMS 4 well plates, Anneal heat to 50°C for 15 min with Rinsing N₂ through oven. React w/ Dye, MMP, Laccase 1 of ~~on~~ on pH 4.5 Wash w/ Solvent (DMF) & Water to remove pharmacies. Retest Best formulations for Reactivity Against Dye.

Samples: All 1% ABN Samples made Std Solution of 1% TMA, GMA

#	Trimethoxylate (TMA)	Glycidyl methacrylate (GMA)
1	10	90
2	20	80
3	30	70
4	40	60
5	50	50
6	60	40
7	70	30
8	80	20
9	90	10
10	95	5
12		
11	100	0

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#	Trimethylolpropane Ethoxylate (14/350/04)	Amt
	4N-acrylate	
A	90	10
B	75	25
C	50	50
D	25	75
E	10	90
F	00	0

Solvented each for, 5 sec
Spotted 1ml onto each slide placed in 70°C oven
in N2 Purge Trough, in Glass Tray 11:09 Am

Added 10ml of 1% CBTPB to each Cocktail 15 ml
all Samples have Swelled
A, B, & C Swelled the most
2 & 3 maybe the least or the Slowest.

All became white when Swollen.

Need to try more Cross-linking or it Cures or
That I have not Cured it long enough or
Front purge w/ N2 well enough
Need to do a Screen of the Urea Acrylates
2 and Have & include time

Time	Pentaerythritol pentacrylate is Star	Trimethylolpropane	Amt	CMA	TMA
High 3kv	~~90~~ TAW 4/20/04	90	TAW 4/20/04	60	10	
Low 15mil		90		Continued on Page	
	90	Read and Understood By		10	
	90	~~90~~ TAW 4/20/04		10	TAW 4/20
	4/20/04				TAW

Signed _____ Date _____ Signed Craig Mayer Date 4/21/04

PROJECT Formulated Resins tree.

Notebook No. _____

Continued From Page 99

Swellable Formulated

↓

Functionalized Acrylate Monomer

Stabilizing cross-linking

Treatment when Swollen

RAPID Curing

non-fluorescent

Easy to wash

↓

NEED Solvent ? ~~Acetonitrile~~ ~~EtOH~~

1:1 NEOA : EtOH

25 % 2-amino methacrylate or Gma

50 % Peg Dimethacrylate

20 % Trimethylolpropane ethoxylate

4 % AIBN

40 to 60 % 2-amino methacrylate or Gma

48 % trimethylolpropane ethoxylate

12 % AIBN

1/20 oil

Yellow

The amino methacrylate is better would be used to dye beads

or could Use Glycidyl MA + Functionalize

yes.

Non Swelling Formulated

↓

- Maximize cross linking
- Functionalize surface
- Transparent
- Rapid Curing
- non fluorescent

↓

No Solvent

↓

90% Di pentaerythritol penta Acrylate

10% Glycidyl MA or enough to flow / dissolve AIBN.

CAS # 6050 6-8

Use

Continued on Page

Signed _____ Date _____

Read and Understood By

Craig Lamaye 4/21/04

Signed Date

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/015764

International filing date: 06 May 2005 (06.05.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/608,774
Filing date: 10 September 2004 (10.09.2004)

Date of receipt at the International Bureau: 20 June 2005 (20.06.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1331929

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APPLICATION NUMBER: *60/608,774*
FILING DATE: *September 10, 2004*
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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Trent Russell	Northen	Tempe, Arizona

Additional inventors are being named on the ___ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

LIGHT ACTIVATED MOVING POLYMER

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☑ Customer Number 26707

OR *Type Customer Number here*

☐ Firm *or* Individual Name

Address

Address

City | State | ZIP

Country | Telephone | Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 12

☐ Drawing(s) *Number of Sheets*

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number

☐ Other (specify) Cover sheet; Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($) $80

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

SIGNATURE Robert D. Atkins

TYPED or PRINTED NAME Robert D. Atkins

TELEPHONE (602) 229-5311

Date 09/10/04

REGISTRATION NO. *(if appropriate)* 34,288

Docket Number: 112624.00147

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This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Patent Application, Commissioner for Patents, Alexandria, VA 22313-1450.

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Applicant:	Trent Russell Northen	
Date of Filing:	September 10, 2004	Art Unit: Unassigned
Title:	*LIGHT ACTIVATED MOVING POLYMER*	Examiner: Unassigned
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1) Provisional Application For Patent Cover Sheet
2) Specification (12 pages, plus cover sheet)
3) Return postcard

PATENT

PROVISIONAL APPLICATION

Of

TRENT RUSSELL NORTHEN

For

UNITED STATES LETTERS PATENT

on

LIGHT ACTIVATED MOVING POLYMER

Attorneys:

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Express Mail Label No.: EL988555789US
Attorney Docket No.: 112624.00147

Light Directed Movement of Polymer Microstructures

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Abstract

Light induced surface chemistry changes have been used move swollen polymer microstructures. Swellable trimethylolpropane trimethacrylate (TRIM) crosslinked poly(2-hydroxylethyl methacrylate) conical microstructures were constructed by azo-bis-isobutyronitrile (AIBN) photopolymerization using a 20x 0.5NA microscope objective and 365nm laser excitation. Stuctures were aminiated with glycine and protected with the photolabile group 4-nitroveratryloxycarbanyl (NVOC). Differential swelling with and without NVOC of 10% was observed in N,N'-dimethylformamide (DMF). Removal of NVOC with 365nm laser excitation induced polymer shrinkage in excess of 4%, resulting in maximum polymer velocity of 1mm/s, and displaced solvent velocities in excess of 0.01mm/s.

Introduction

A number of synthetic polymers have recently been developed that respond to changes in surface energy resulting from external stimuli including: mechanical deformation, heating, solvent contact, and exposure to light[1]. Exciting applications of such materials include: implants based on shape-memory materials, gels respond (e.g. swell) in response to changes in pH or specific molecules may be used for feedback control for drug delivery, and microfabricated vascular networks[2]. Given the fact that three-dimensional polymer structures can now be constructed on the submicron scale using nonlinear laser patterning [3, 4] [5, 6], it should also be possible to develop micro or nanomechanical devices based on polymer movement.

A particularly versatile stimulus that could be used for directing polymer movement at dimensions down to the submicron level is light. Photolabile protective groups offer the ability to selectively break bonds using light and therefore substantially change the surface characteristics of the polymer in a light-directed fashion. 4-nitroveratryloxycarbanyl (NVOC) is a common photolabile group and is known to cleave using a Norrish-type II reaction [7]. It has found wide use in protecting amines [8] and has applications including: photogeneration of organic bases [9], microarrays [10], novel proteins [11], and variations of NVOC as linkers in peptide synthesis [12]. In these cases the addition and removal of NVOC modulates the reactivity of an amine. This work describes the use of NVOC to instantaneously modulate the surface properties of a porous polymer using light.

Porous polymers are common in solid phase synthesis [13], drug delivery [14, 15], tissue engineering [16], and separations [17] [18]. A range of polymers are now used for solid phase synthesis including polyacrylate resins [19]. These porous polymer structures are the result of phase separation during free radical crosslinking copolymerization swell in

compatible solvents [20]. Typically solvents (porogens) can be used to control the pore size[21]. The surface of the polymer is often modified to improve functionality [22] or as a result of solid phase synthesis.

Here we report polymer microstructures that shrink and move when illuminated with light. Cleavage of NVOC immediately exposes the primary amine, resulting in large changes in the surface chemistry and swelling of the polymer. This allows light-directed spatial control of polymer movement.

Experimental Details

Materials: Glass coverslips for an FCSII chamber (see below) were purchased from Bioptechs (Butler, PA). 2-hydroxylethyl methacrylate (HEMA), trimethylolpropane trimethacrylate (TRIM), azo-bis-isobutyronitrile (AIBN), piperidine, diisopropylethylamine were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). 4-nitroveratryloxycarbanyl chloride and 3-(trimethoxysilyl)propyl methacrylate were from Fluka GmbH (Buchs, Switzerland). Dimethylformamide (DMF) was from Applied Biosystems Inc. (Foster City, CA). Methanol, hydrogen peroxide (30%), sulfuric acid, hydrochloric acid were purchased from Mallinckrodt Inc. (Paris, KY). Isopropanol and ethanol were from ACROS Organics (Geel, Belgium). Acetonitrile was from Alfa Aesar (Ward Hill, MA). 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and FMOC-glycine (FMOC-Gly) were from Advanced ChemTech Inc. (Louisville, KY). Finally, water was purified using a NANOPure ultrapure filtration system Barnstead. (Dubuque, IA).

Equipment: All reactions were performed inside an FCSII flow chamber Bioptechs Inc. (Butler, PA). Patterning and deprotection were done using light from a mode-locked Tsunami Ti:sapphire laser pumped by a 5 W Millennia Vs diode-pumped cw laser, Spectra-Physics Inc.(Mountain View, CA), through a 20x 0.5NA objective attached to an Eclipse TE2000-U microscope, Nikon Inc.(Japan) equipped with a ProScan microscope stage, Prior Scientific Inc.(Rockland, MA). The laser beam was modulated using a Model 350-80 electro-optic light modulator with model 302 power supply, Conoptics Inc. (Danbury, CT) controlled by software developed in-house. Laser power was measured using a Model 1815-C power meter, Newport Co. (Irvine, CA). Images taken using Cascade Photometrics CCD, Roper Scientific Inc. (Tucson, AZ) through 10x 0.3NA objective lens, Nikon Inc. (Japan) using MetaVue 6.0 software, Universal Imaging Corporation Limited (Marlow, UK) for acquisition and analysis. Scanning electron microscopy (SEM) was performed using a XL30ESEM environmental SEM, FEI Co. (Hillsboro, OR) on a sample coated with 3.5nm palladium /gold.

Surface Functionalization. Glass cover slides for a FCSII flow chamber were cleaned using a modification of methods reported by McGall [23]. Briefly: slides were soaked 15 min at RT with 60/40 (v/v) sulfuric acid/hydrogen peroxide (use extreme caution when using this solution), placed in 10% sodium hydroxide (w/v) at 70°C for 3 min and placed in 1% HCl at RT for 1 min. Between each step the slideds were soaked in nanopure water for 3 minutes. A solution of 1% 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol 5 % water was mixed for 10 minutes, and the slides were reacted at RT for 15 minutes with gentile agitation. Slides were then soaked in isopropyl alcohol for 3 min, nanopure water for 1 min, and then placed in a 100°C oven for 5 minutes after which the oven was turned off and nitrogen was blown through for 1 hr. The slides were stored under nitrogen until they were used.

Light Directed Movement of Polymer Microstructures

Fabrication of Polymer Structures. A total of 6mg of AIBN was dissolved in 95 µL HEMA and 579 µL TRIM. This was placed in an optical chamber, and irradiated with 4 mW (all powers reported are measured entering microscope) of 365nm (8nm full-width-at-half-maximum) light for 1.6s per feature through a 20x objective focused 400 µm above the surface of the cover slip. Excess monomer was drained and sample washed with methanol and DMF. The features were spaced 600 µm apart..

Amination of Microstructures. FMOC-Gly was coupled to the photopolymer hydroxyl group using 18.6mg FMOC-Gly, 22.5mg HBTU, 11.5 µL DIPEA, and 600ul DMF. Reaction was mixed at 50°C for 30 min. The structures were then rinsed with DMF and the FMOC removed with 20% piperidine in DMF for 10min. The yield of the reaction was determined using the absorbance at 301nm for the FMOC-piperidine adduct. Typical polymer substitution levels were 0.1 nanomoles/feature.

Coupling NVOC and 6-nitrophenyl chloroformate (NPC) to aminated microstructures. A solution of 19mg NVOC or 14mg NPC, 40µl DIPEA, and 600µl DMF was reacted with polymer microstructures by mixing for 30min at 50°C.

Laser cleavage of NVOC: The same laser beam used for making the microstructures was used for cleavage of the NVOC. The beam was attenuated as needed.

Swelling and Tip Velocity Measurements. Images taken of the microstructures at the glass polymer interface in various solvents were manually fitted with ellipses of known pixel area. Tip velocity was calculated from the distance moved in sequential images over a known amount of time.

Results and Discussion

Porous polymer microstructures. Polymer structures were obtained via the photopolymerization of HEMA and TRIM with AIBN. Oxygen quenching[6] and light

Light Directed Movement of Polymer Microstructures

attenuation from AIBN absorption were used to limit the polymer structure dimensions to the volume of excitation between the surface and the focus of the laser.

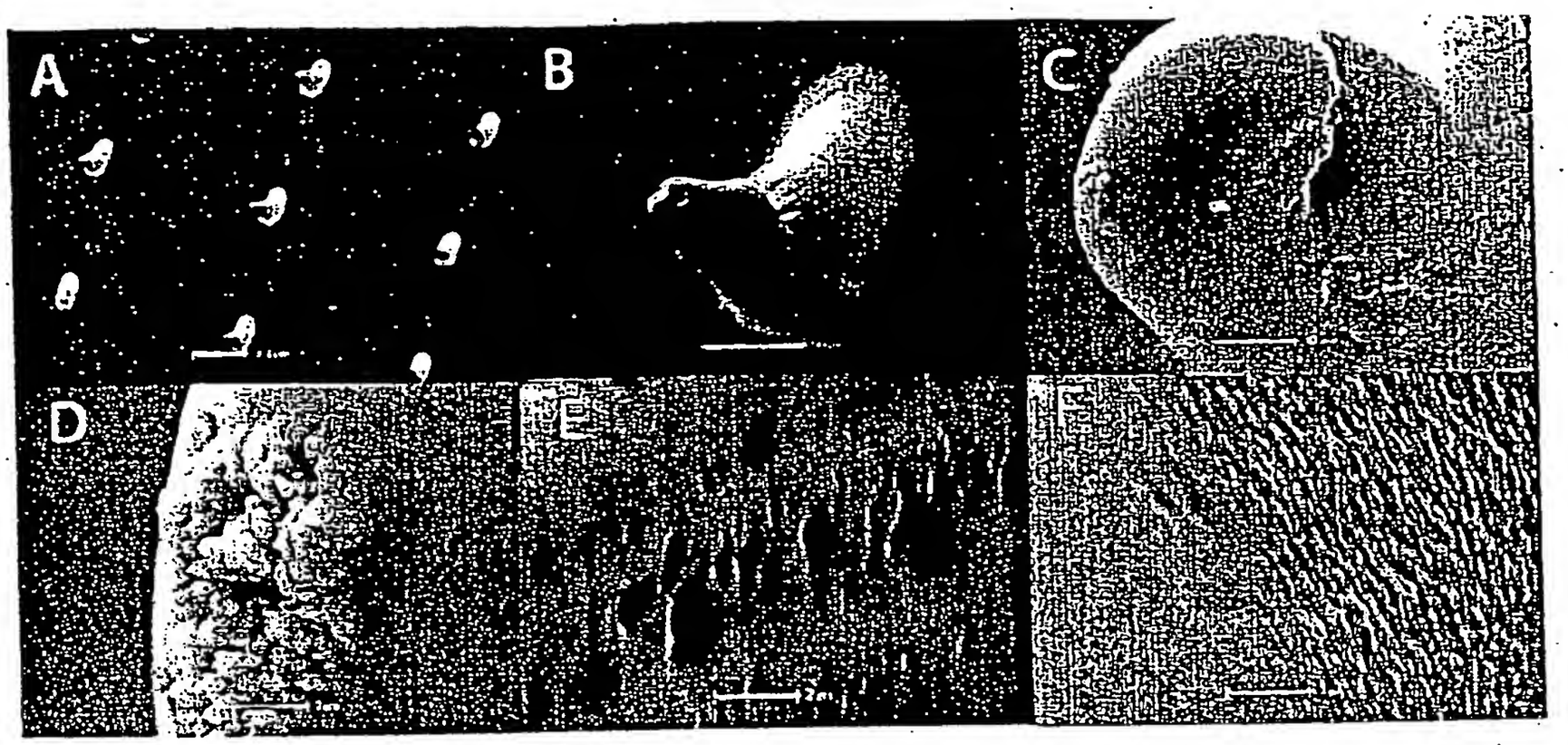


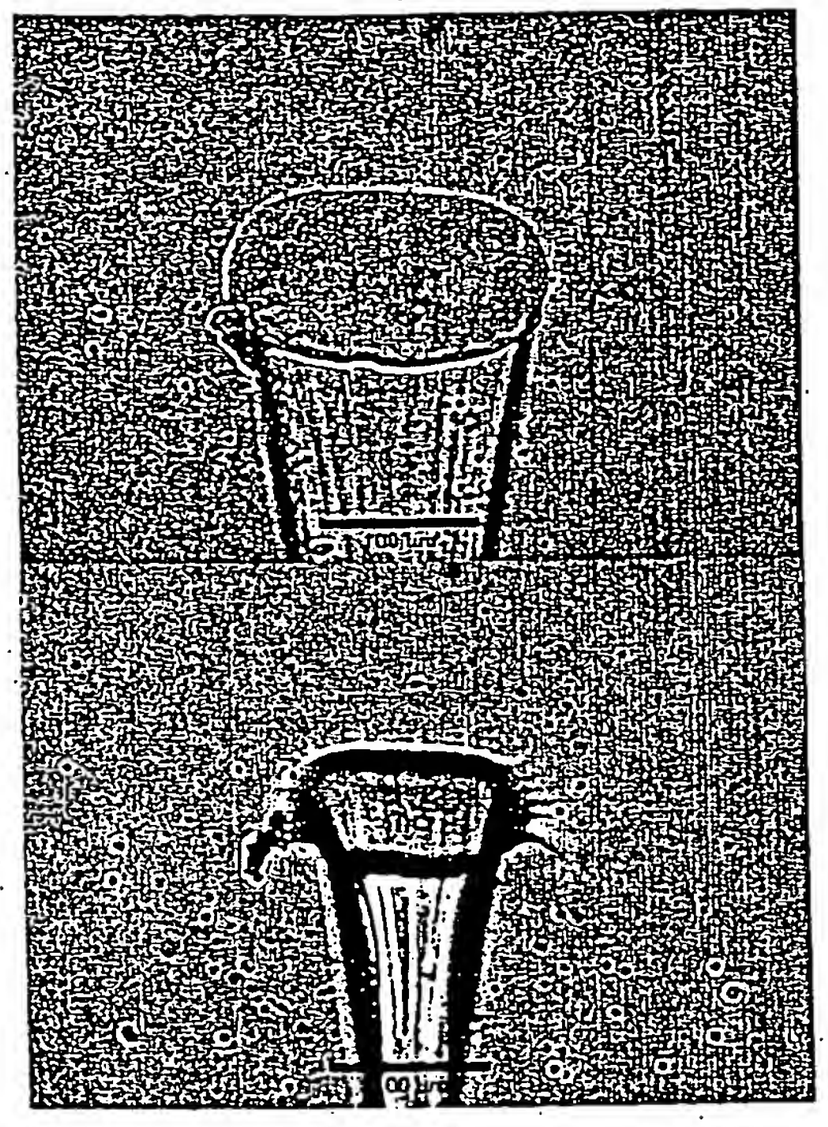
Figure1. SEM images of polymer microstructures: (A) a portion of the array, (B) one microstructure, (C) top of microstructure, (D) macropore at tip of microstructure, (E) macropores below tip, (F) junction of smooth central region with lowest rough/microporous region.

An array of polymer features was generated by laser-directed photopolymerization (Figure 1A). Partial polymerization results in soft porous structures that were measured on the optical microscope to be 400 μm tall, having an elliptical base with radii of 75 and 200 μm. The structure of each feature has a heterogeneous morphology due to spatial differences in light intensity in the focused laser beam. Structures appear composed of four regions (Figure 1B): two macroporous regions close to the beam focus (Figure 1 C-E), an apparently nonporous central region (smooth area in Figure 1 F), and a rough potentially porous region nearest the glass surface (rough area in Figure 1 F). The macropores at the top are on order of 1 μM. The number of reactive sites (using FMOC as a probe) was estimated to be 0.1 nmole per feature, 5 orders of magnitude more than would be expected for a nonporous material.

Solvent Swelling: The swelling of the polymer with and without the NVOC protective group was measured in various solvents. It was found that the greatest swelling, and largest difference in swelling between the protected and unprotected resin, was in the polar aprotic solvents DMF and acetonitrile (Figure 2). The swelling of the polymer was found, as expected, to be related to

Light Directed Movement of Polymer Microstructures

the Hildebrand solubility parameter [24] where maximum swelling of a slightly crosslinked polymer occurs in solvents of similar solubility parameter [24].



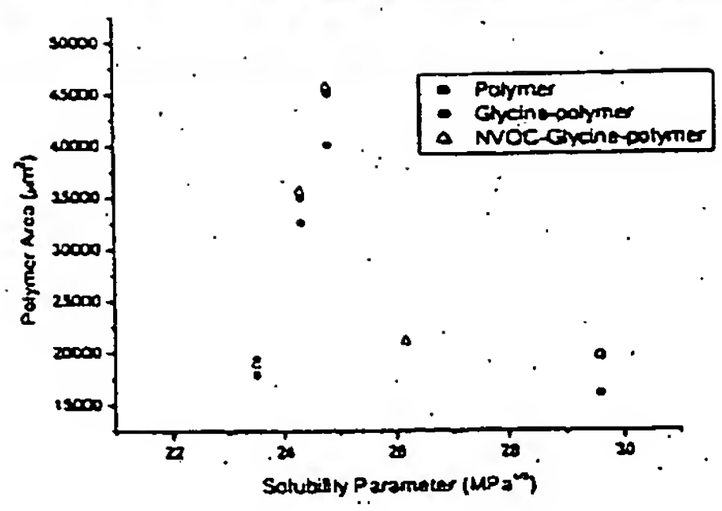


Figure 2. (top) Images of NVOC-Gly polymer features laying on the glass surface, illustrating the difference in swelling when exposed good solvents (acetonitrile) and poor solvents (methanol). The images were recorded using a 10x objective lens focused at polymer glass

Light Directed Movement of Polymer Microstructures

interface. (bottom) swelling of polymer, Glycine-polymer, NVOC-Gly polymer in various solvents as a function of the Hidebrand parameter.

It is apparent from Figure 2 that the swelling of the resin changes dramatically with the solvent. In DMF the area of the NVOC-Gly polymer at the glass polymer interface increased 10% versus the Gly polymer. This has been seen before; it was found by Merrifield that over the course of solid phase peptide synthesis, resin swelling increased more than five fold [25]. This behavior was attributed to the net decrease in free energy upon swelling due to solvation of peptide chains bound to the polymer matrix. Presumably a similar solvation mechanism accounts for the differential swelling of the resin with and without NVOC as shown in figure 2.

Polymer Movement. Upon laser excitation of the NVOC-Gly polymer structures in DMF or acetonitrile, the NVOC is photocleaved, resulting in shrinking of the illuminated portion of the polymer, causing the polymer to bend. Figure 3 shows a series of images collected as a polymer structure moves towards the laser beam.

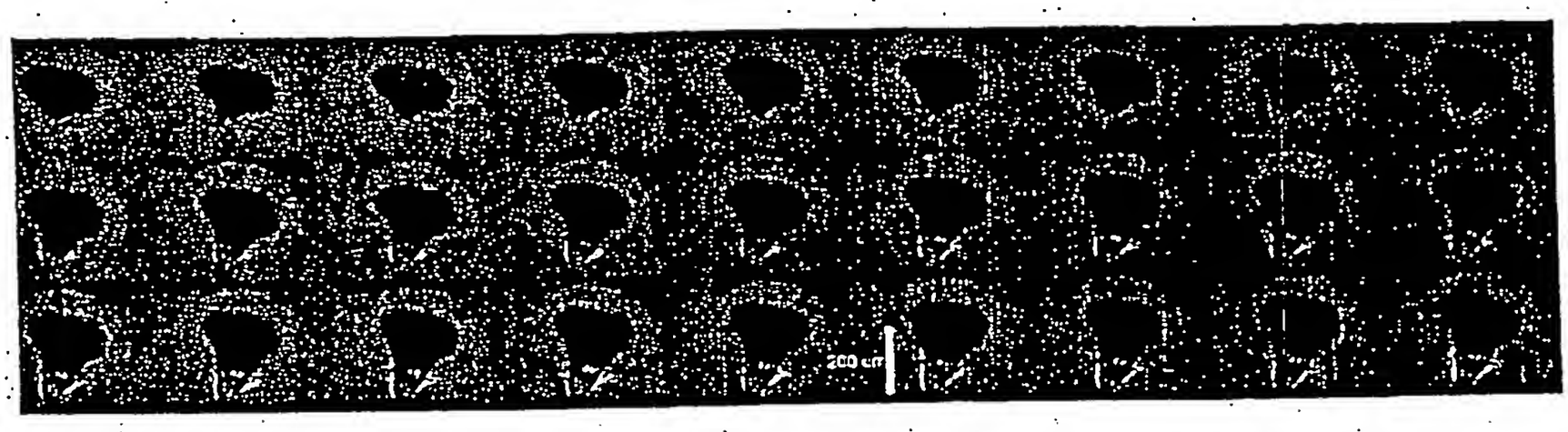
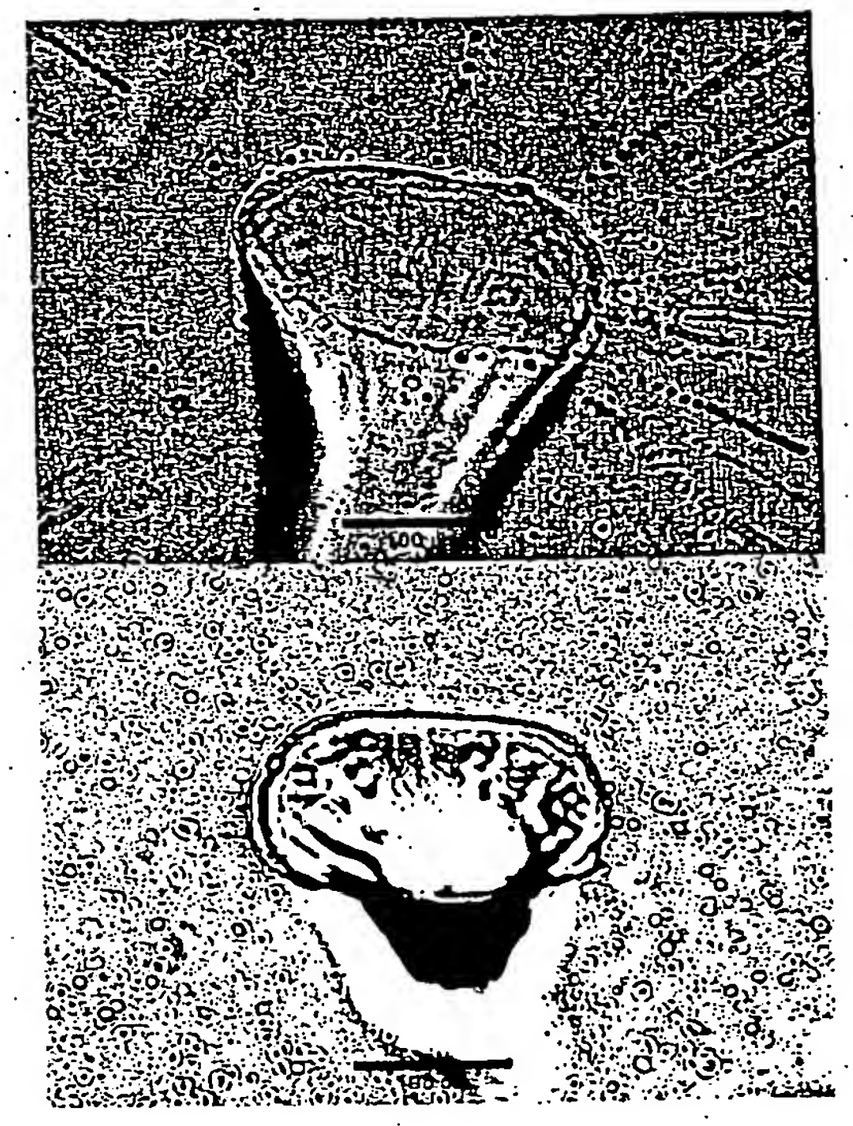


Figure 3. Progression of images of NVOC-Gly polymer structure with asymmetric illumination at lower edge of polymer structure. Perspective is looking down on the polymer as it bends toward the lower edge of the images. The upper left image of the series represents the point at which illumination was initiated, with time continuing from left to right and then down the array. Images were taken through a 10x objective lens at 5ms per frame and the solvent used was acetonitrile.

Light Directed Movement of Polymer Microstructures

Symmetric illumination of an NVOC-Gly polymer feature results in the rapid release of solvent from the microstructure. Small particulates in solution rapidly move radially away from the polymer structure during shrinkage with a maximum velocity of 0.01mm/s. This provides a lower limit for the maximum fluid velocity. It should also be noted that the NVOC group cleaves as nitrosobenzyl aldehyde [Patchornik, 1970 #1] which is released upon illumination. A modified photocleavable group of this nature could be used as a method for local delivery of reagents or drugs.

The base area of the polymer shrinks by ~4% after a 20sec 400uW illumination period (Figure 4 top and bottom respectively). Given that the polymer is covalently attached to the glass it is expected that the actual bulk shrinkage is greater than is observed at or near the glass surface.



Light Directed Movement of Polymer Microstructures

Figure 4. (top) Movement of particles away from polymer upon illumination with light. (bottom) Difference image showing shrinkage of polymer structure upon laser excitation, before (black) and after (white) 20sec of 400 μW

The polymer movement is very rapid especially at the tip of the polymer structure. Velocities on order of 1mm/sec were recorded (Figure 5) in acetonitrile. This is several times faster than that observed in DMF (Figure 5) even though the differential swelling is larger in DMF (Figure 1). This is explained by the three fold higher viscosity of DMF resulting in greater resistance to flow, slowing the movement of the microstructure. It is also clear that there was little if any movement of structures in solvents that did not swell the resin (Figure 5).

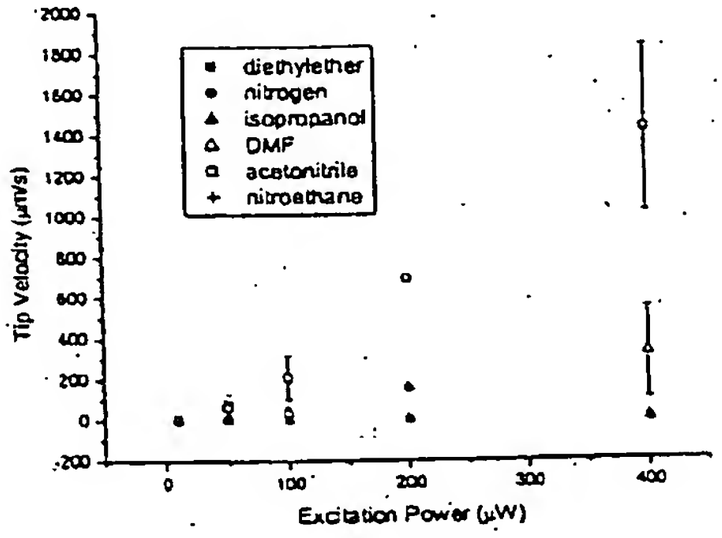


Figure 5. Tip velocity as a function of excitation power in various solvents.

Chemical mechanism of polymer volume change upon illumination: The design hypothesis underlying the development of this system was that photocleavage induced changes in surface chemistry result in the volume changes and associated polymer movements. The other possible mechanisms for mechanical movement upon illumination and photocleavage include photochemical curing processes, electrostatic forces, hydrogen bonding, and optical trapping, and measurements were performed to investigate each of these.

A photochemical curing process is unlikely for several reasons. The polymer movement continues for ~1sec after a 100ms 1mW exposure in DMF. Given the presence of oxygen and low viscosities of the solvents it is unlikely that any free radical or photochemical process would continue for this long in the dark. It was also found (Figure 6) that these dramatic movements

only occurred with NVOC and not in the presence of the polymer itself or with another chromaphore (NPC). It was possible to reattach NVOC and regain partial polymer movement.

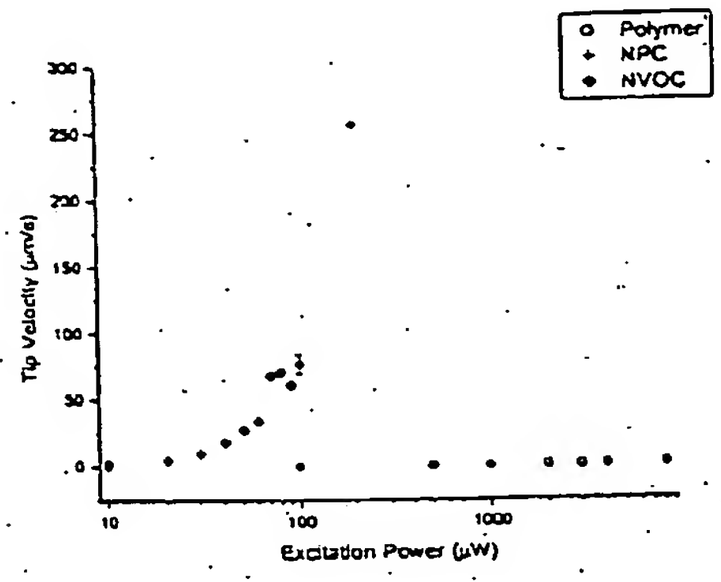


Figure 6. Tip velocity as a function of input power for polymer structures in DMF.

To test for the role of hydrogen bonding of the hydroxyl or amine groups, the polymer was soaked for approximately 1 hour in acetic anhydride and then illuminated. While this reduced the speed of tip movement (50 µm/s with 400µW excitation), the movement was still substantial, showing that the movement is not a result of hydrogen bond formation upon NVOC cleavage since all hydroxyls and amines should have been rapidly acylated in acetic anhydride.

The fact that the polymer curves towards the laser beam makes an electrostatic mechanism (formation of protonated amine groups or charged intermediates in the photocleavage reaction) unlikely since one would expect repulsive electrostatic forces to push the polymer away from the illuminated region (Figure 3). Additionally, the movement was not observed in nonpolar solvents, where the electrostatic effect should be greatest and was observed even in a solvent system with a 60 mM ionic strength that should strongly shield the charge-charge interactions (data not shown). Finally, the movement of the polymer is not dependent on the position of the focus, making optical trapping a very unlikely mechanism. Polymer movement is observed even when the focus is at the polymer glass interface, 400 µm from the polymer tip.

Conclusion

We have described porous polymer structures that make dramatic movements with the rapid release of solvent when illuminated. We hope to attach spiropyrans to these polymer structures in attempt to make a reversible system. This may find use in systems where it is desirable to control the movement of a polymer structure or for releasing material into solution. Such a system would be very exciting for converting light energy into mechanical movement.

Acknowledgements

Sudhir Gudala for developing the scanning software. Saritha Keshammolu for surface preparation.
Center for Solid State Science at Arizona State University for SEM

References

1. Russell, T.P., *Surface-responsive materials*. Science, 2002. 297(5583): p. 964-967.
2. Langer, R. and D.A. Tirrell, *Designing materials for biology and medicine*. Nature, 2004. 428(6982): p. 487-492.
3. Kawata, S., et al., *Finer features for functional microdevices - Micromachines can be created with higher resolution using two-photon absorption*. Nature, 2001. 412(6848): p. 697-698.
4. Sun, H.B., et al., *Three-dimensional nanonetwork assembled in a photopolymerized rod array*. Advanced Materials, 2003. 15(23): p. 2011-2014.
5. Belfield, K.D., et al., *Multiphoton-absorbing organic materials for microfabrication, emerging optical applications and non-destructive three-dimensional imaging*. Journal of Physical Organic Chemistry, 2000. 13(12): p. 837-849.
6. Maruo, S. and K. Ikuta, *Three-dimensional microfabrication by use of single-photon-absorbed polymerization*. Applied Physics Letters, 2000. 76(19): p. 2656-2658.
7. Bochet, C.G., *Photolabile protecting groups and linkers*. Journal of the Chemical Society-Perkin Transactions 1, 2002(2): p. 125-142.
8. Patchornik, A., B. Amit, and R.B. Woodward, *Photosensitive protecting groups*. Journal of the American Chemical Society, 1970. 92(21): p. 6333-5.
9. Cameron, J.F. and J.M.J. Frechet, *Photogeneration of Organic-Bases from Ortho-Nitrobenzyl-Derived Carbamates*. Journal of the American Chemical Society, 1991. 113(11): p. 4303-4313.
10. Fodor, S.P.A., et al., *Light-Directed, Spatially Addressable Parallel Chemical Synthesis*. Science, 1991. 251(4995): p. 767-773.
11. Mendel, D., J.A. Ellman, and P.G. Schultz, *Construction of a Light-Activated Protein by Unnatural Amino-Acid Mutagenesis*. Journal of the American Chemical Society, 1991. 113(7): p. 2758-2760.
12. Holmes, C.P. and D.G. Jones, *Reagents for Combinatorial Organic-Synthesis - Development of a New O-Nitrobenzyl Photolabile Linker for Solid-Phase Synthesis*. Journal of Organic Chemistry, 1995. 60(8): p. 2318-2319.
13. Merrifield, R.B., *Solid Phase Peptide Synthesis .1. Synthesis of a Tetrapeptide*. Journal of the American Chemical Society, 1963. 85(14): p. 2149-&.
14. Kost, J. and R. Langer, *Responsive Polymer Systems for Controlled Delivery of Therapeutics*. Trends in Biotechnology, 1992. 10(4): p. 127-131.
15. Langer, R., *Drug delivery and targeting*. Nature, 1998. 392(6679): p. 5-10.
16. Liu, V.A. and S.N. Bhatia, *Three-dimensional photopatterning of hydrogels containing living cells*. Biomedical Microdevices, 2002. 4(4): p. 257-266.
17. Svec, F., et al., *Design of the monolithic polymers used in capillary electrochromatography columns*. Journal of Chromatography A, 2000. 887(1-2): p. 3-29.
18. Righetti, P.G., *Macroporous Gels - Facts and Misfacts*. Journal of Chromatography A, 1995. 698(1-2): p. 3-17.
19. Kempe, M. and G. Barany, *CLEAR: A novel family of highly cross-linked polymeric supports for solid-phase peptide synthesis*. Journal of the American Chemical Society, 1996. 118(30): p. 7083-7093.

20. Okay, O., *Macroporous copolymer networks*. Progress in Polymer Science, 2000. 25(6): p. 711-779.
21. Yu, C., et al., *Preparation of monolithic polymers with controlled porous properties for microfluidic chip applications using photoinitiated free-radical polymerization*. Journal of Polymer Science Part a-Polymer Chemistry, 2002. 40(6): p. 755-769.
22. Rohr, T., et al., *Photografting and the control of surface chemistry in three-dimensional porous polymer monoliths*. Macromolecules, 2003. 36(5): p. 1677-1684.
23. McGall, G.H., et al., *The efficiency of light-directed synthesis of DNA arrays on glass substrates*. Journal of the American Chemical Society, 1997. 119(22): p. 5081-5090.
24. Barton, A.F.M., *Solubility Parameters*. Chemical Reviews, 1975. 75(6): p. 731-753.
25. Sarin, V.K., S.B.H. Kent, and R.B. Merrifield, *Properties of Swollen Polymer Networks - Solvation and Swelling of Peptide-Containing Resins in Solid-Phase Peptide-Synthesis*. Journal of the American Chemical Society, 1980. 102(17): p. 5463-5470.

This invention can be used as _ This technology would allow the conversion of light energy to mechanical energy either through the movement of a fluid or the polymer itself. There would seem to be a wide range of possibilities for such a material. Ranging from large solar collectors, light powered nanobots (photopolymer structures 100's of nanometers can be readily made), artificial muscle, drug delivery systems, microfluidic pumps and valves, etc.

1. This invention provides the following advantages: It results in dramatic changes in the physical dimensions of the polymer.
2. It releases (or could absorb) solvent
3. It is a general system that could be used with any porous polymer formulation or potentially on the surface of very thin polymer structures.
4. The surface area of a porous polymer is many orders of magnitude higher than a non porous polymer, this would be the preferred mode for drug delivery

___ There are molecules (Azobenzene, spiropyrans, etc) that act as molecular switches, one color of light puts them in one form, another moves them back to the initial form. By attaching one of these molecules to the surface that has a large polarity change upon switching forms, it should be possible to make a polymer that expands with one color of light and contracts with another color of light. This technology would allow the conversion of light energy to mechanical energy either through the movement of a fluid or the polymer itself. There would seem to be a wide range of possibilities for such a material. Ranging macroscale solar collectors, light powered nanobots (photopolymer structures 100's of nanometers can be readily made), artificial muscle, drug delivery systems, microfluidic pumps and valves, etc.

There is a NVOC derivative that is used as a linker in peptide synthesis. It could be used to release a material of interest. One end of the linker would be linked to the polymer, the other to the material (drug) to be released. By adjusting the surface energy of the polymer it would be possible to design a system that would rapidly release the material with light. Merrifield has shown that polymer resin swells 5x with a large peptide attached—this system with a photocleavable linker would allow the delivery of peptide (and other) drugs accompanied with a rapid movement of solvent.

It may be possible to use a conducting polymer and switch the polymer states by oxidizing and reducing groups on the surface electrically. This would allow this technology to be used in places not accessible to light (inside the body) or in electrical devices.

There are other photoactivated groups and polymers that could be used.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/015764

International filing date: 06 May 2005 (06.05.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/623,181
Filing date: 29 October 2004 (29.10.2004)

Date of receipt at the International Bureau: 20 June 2005 (20.06.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1331930

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

June 09, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/623,181*
FILING DATE: *October 29, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/15764*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

(09/04)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL988555877US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Trent Russell Neal Walter	Northen Woodbury	Tempe, Arizona Tempe, Arizona

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

PEPTIDE CHARACTERIZED FOR PATTERNED PHOTOPOLYMER FORMED USING LIGHT DIRECTED SYNTHESIS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 26707

OR

☐ Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 21

☐ Drawing(s) *Number of Sheets*

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number of CDs

☑ Other (specify) Cover Sheet; postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

FILING FEE Amount ($): 80.00

☐ Payment by credit card. Form PTO-2038 is attached.

☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055
A duplicative copy of this form is enclosed for fee processing.

☑ invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

SIGNATURE Robert D. Atkins

Date October 29, 2004

TYPED or PRINTED NAME Robert D. Atkins

REGISTRATION NO. 34,288 *(if appropriate)*

TELEPHONE 602-229-5311

Docket Number: 112624.00138

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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1882295

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Date of Signature and Deposit: October 29, 2004

By: Maritza O'Neill
(Signature of person depositing mail)

MARITZA O'NEILL

CERTIFICATE OF MAILING PURSUANT TO 37 C.F.R. 1.10

Applicant:	Trent R. Northen *et al.*	
Date of Filing:	October 29, 2004	Art Unit: Unassigned
Title:	*PEPTIDE CHARACTERIZED FOR PATTERNED PHOTOPOLYMER FORMED USING LIGHT DIRECTED SYNTHESIS*	Examiner: Unassigned
Attorney Docket No.: 112624.00138		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Type of Filing:

1) Provisional Application For Patent Cover Sheet
2) Specification (21 pages, plus cover sheet)
3) Return postcard

PATENT

PROVISIONAL APPLICATION

of

TRENT RUSSELL NORTHEN
NEAL WALTER WOODBURY

For

UNITED STATES LETTERS PATENT

on

PEPTIDE CHARACTERIZED FOR PATTERNED PHOTOPOLYMER FORMED USING LIGHT DIRECTED SYNTHESIS

Attorneys:
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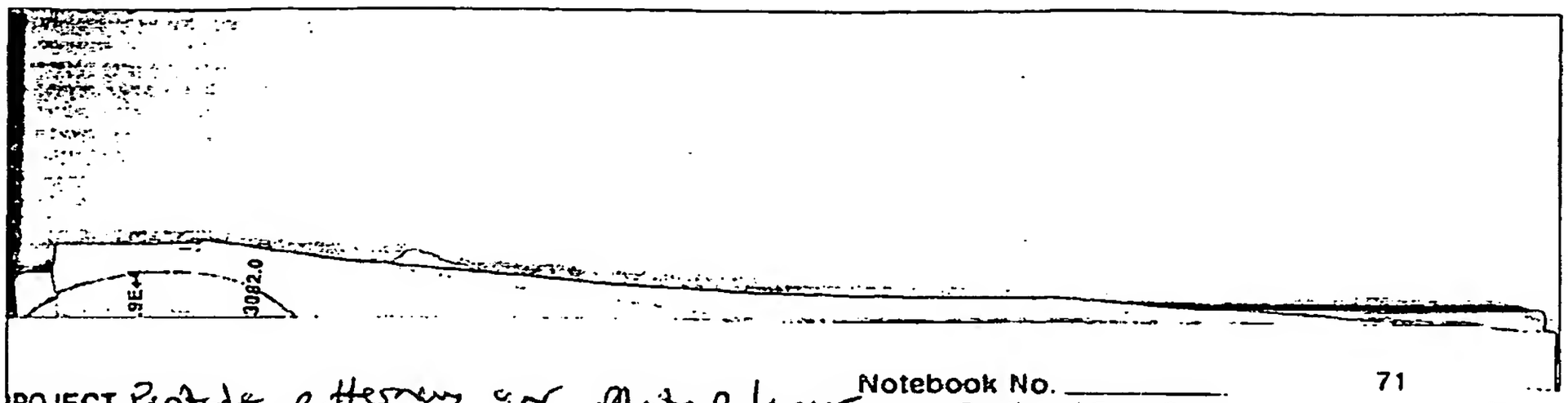
TWO NORTH CENTRAL AVENUE
PHOENIX, AZ 85004-2391

Express Mail Label No.: EL988555877US
Attorney Docket No.: 112624.00138



PROJECT Peptide patterning on Photopolymer Notebook No. 71
Continued From Page

Purpose: to pattern Fmoc-6-6-F-L-CO2H on the polymer
The Reprotect of Nboc & pattern
P S V
Hydrazide Antibody & detect via fluorescence.

Prep-Ex 29 95 mL Hexane + 572 mL THF + 4 mg ABN
Washed & Sonicated with dissolved
added to Chamber of Fourth Multicycle Set.2
Printed at the Page 29 200 730/2 8 nm FWHM 8 mW 25cc 525 nm
2 x 27 x 13 arrays, looks great!

Prep to the Fmoc-G-6-F-L-CO2H PREP-ED By ABN. STM
dissolved in TFA/TIS/Phenol Solution. 100 mL - 1.1 = 80 mg
Extracted w/ Hexane/Ag Kolt w/ (60 mL DIPEA in
Hexane Unnecessary) Dried down Kim Lager (yellow)
80 mg - 60 mL (.742 mg/mL) = 35.5 mg peptide

Fmoc-6-6-F-L-CO2H

Fmoc 223.3 + 2(57.05) + 147.18 + 113.16 + 17 = 614.74 mg/mmole

35.5 mg / 614.74 mg/mmole (= 0.058 mmole) × (2.5 mL / 2.5) = 5.5 x 10⁻² mmole HBTU

× 5 = 0.29 mmole DIPEA

5.5e-2 mmole (379.25 mg/mmole) = 20.9 mg HBTU

0.29 mmole (129.25 mg/mmole) (1.4 mL/mg) = 52.5 µL

per 9.11.04 per 9.15.04
30 mM (614.74 mg/mmole) = ~~9.22 mg~~ 9.5 mg peptide
1000 mL

30 mMole (379.25 mg/mmole) = 5.68 mg HBTU
1000 mL mL

60 mMole (129.27 mg/mmole) (0.742 mg/mL) = 5.6 µL DIPEA
1000 mL

Use 150 mMole 3.1 µL

Continued on Page 72

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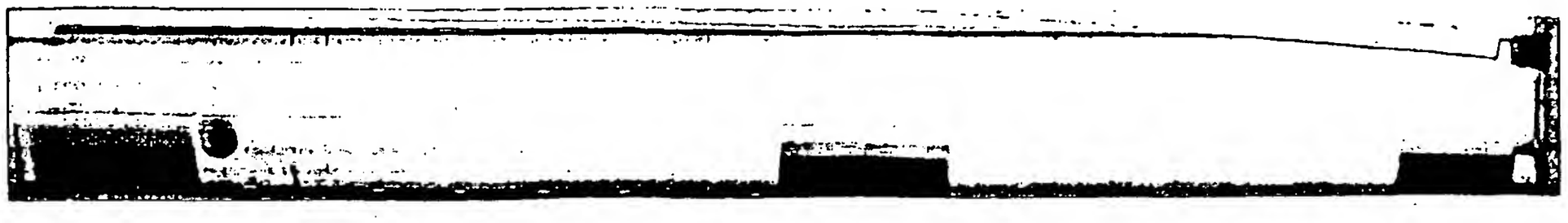
9.15.04 Date — Signed

10/29/04 Date — Signed



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PROJECT Pathway profile on photofilm Notebook No. _____
Continued From Page _____

Solvent procedure from TEJ 8-71

Pathway Lab Sheet

(1) Rinsed 3x rinsed 3x DMF

Rack/HBSS/DMF 11:08am - 12:09 pm 50°C 150 rpm

1hr @ 50°C DMF, 3x DMF 10mm DMF OD_{301} = 0.053

10 mm Pancreatin OD_{301} = 4 x 1.24 = 4.96

(2) Rinsed 4x DMF

Fmoc Y66 FL Coat ~ 15µ / 13N1 DMF / 5.4mg HBTU / 6uN1 DMF

2pm 9.29.04 50°C 150 Rpm

(3) 4pm rinsed 2x @ 50°C 2x (3x DMF)

(4) 10mm Bank OD_{301} = 0.078

(5) 10mm pancreatin OD_{301} = 1.7

(6) 3x DMF

(7) 14mg NVoc Y=mL DMF 600uL DMF 6pm 150Rpm 50°C

(8) Rinsed 3x DMF 7pm left in DMF overnight

(9) 830 pm 9/30 Rinsed 3x DMF 3x ACN

Added 600uL ACN + 200uL DI/DA

Lost as before 730/2 3mm Fwhm

adjust to 2mW right w/ 200 Do 1 Sec exposure

for Lower Arm

It was observed that the Resin no longer Swelled

well in Acetonitrile, w/ 200 but was a Beta

to Rinse w/ ACN 70823

Washed 3x w/ DMF After DMF AREA = 150471

Now 2x. Afterwards

(10) Rinsed 3x ~~DMF~~ ACN 3x DMF, 3x ACN

Dried w/ N2

Continued on Page

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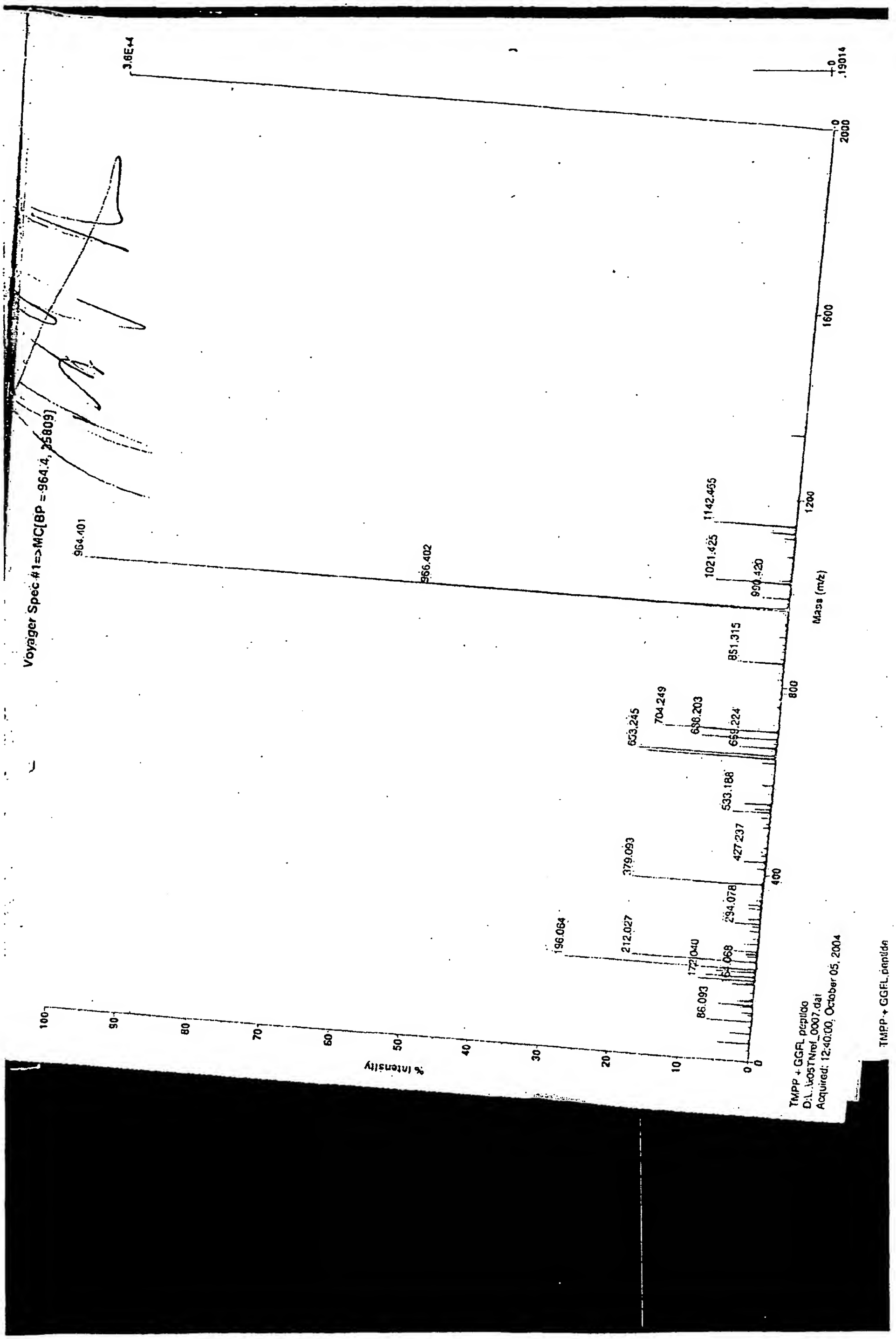
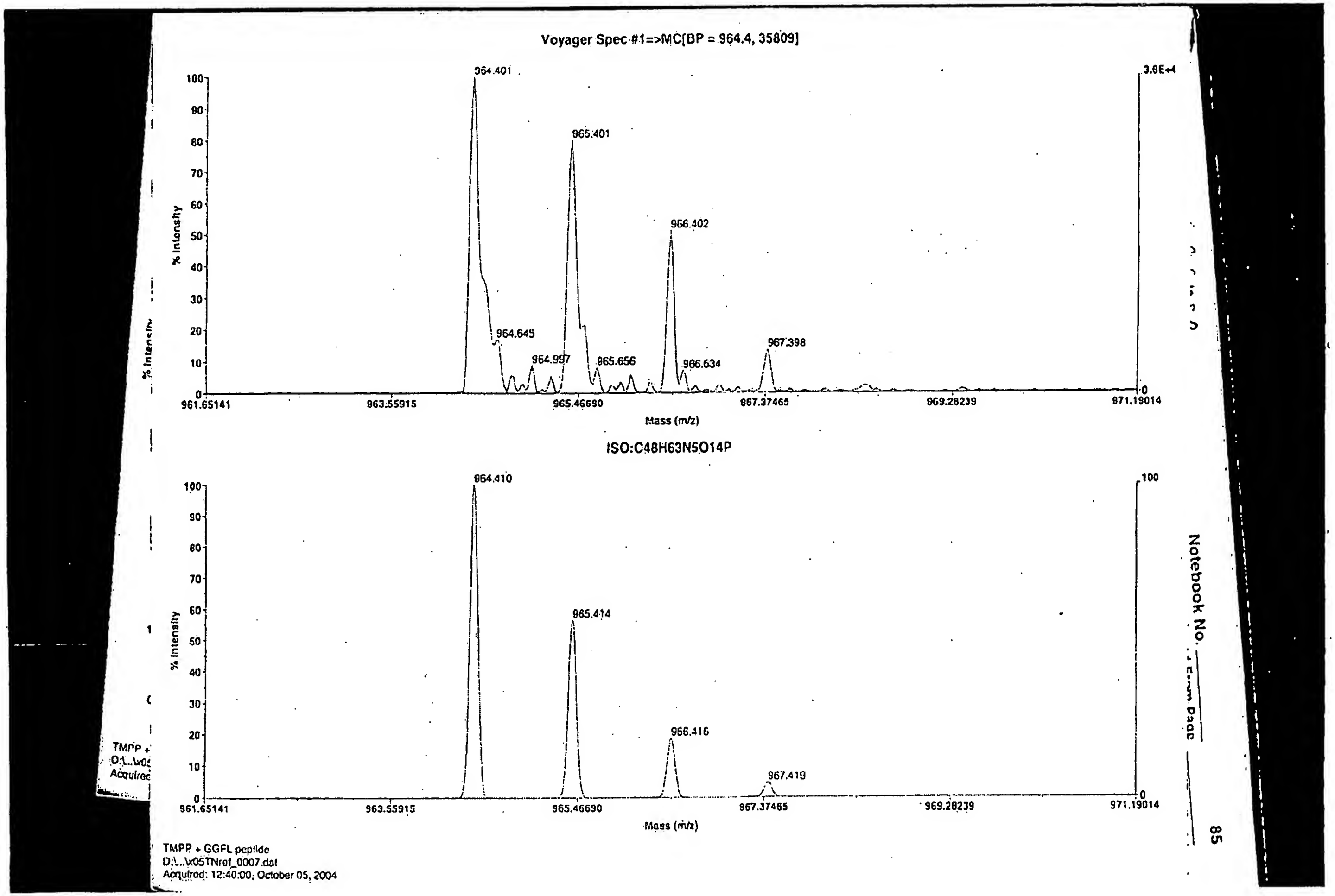
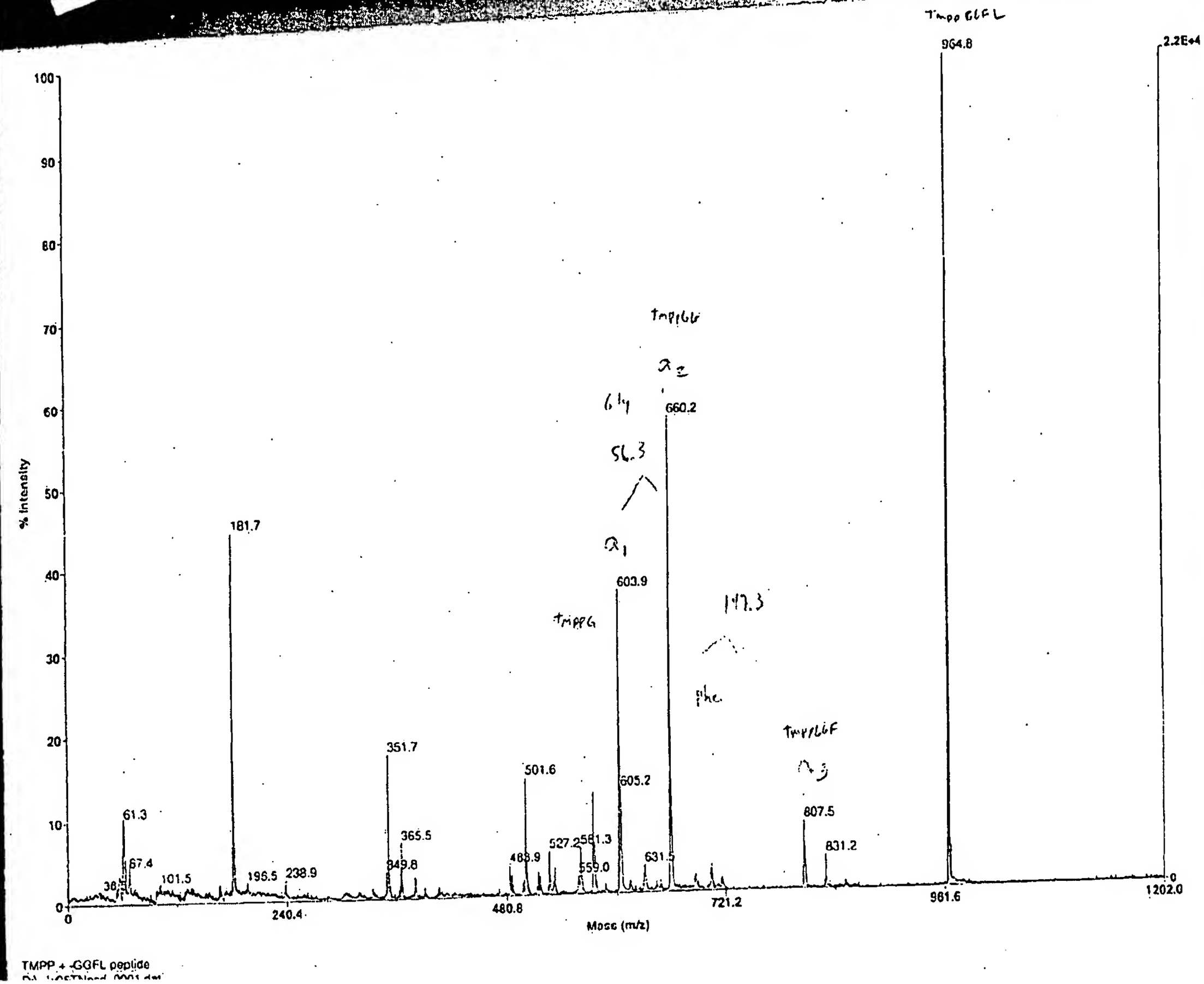




Notebook No. _____ Page _____

85

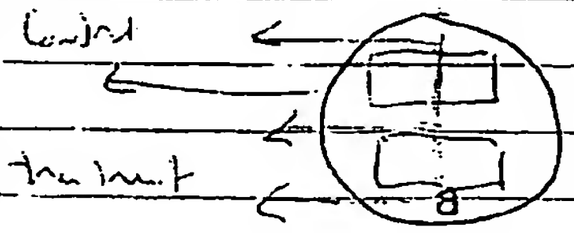
4



TMPP + GGFL peptide

PROJECT GRGDSP

Notebook No. _____ 85
Continued From Page _____



$\frac{(1\,mM)}{100\,mL}\left(\frac{76.8\,mg}{mmole}\right) = 0.0768\,mg$ TMPP-Ac-OBut Br⁻ from D Brune Ref Anal Biochem 268 305-317 1999

5x = 0.384 mg

~ 0.5 mg TMPP-Ac-OBut Br⁻ + 20 mL DIPEA + 980 mL DMF

10 am stirred @ 35°C 150 RPM

(2) 11 to 2 pm Rinsed 3x DMF left @150 rpm 35°C - 1 pm

Rinsed 3x more ! Removed ½ of each array

placed in Eppendorfs w/ 120µl 2 TRIS Hydrogel Slime/TFA

Sonicated for 15 min ! Light exposed second to 5th

after more than an hour.

Concentrated ! Gave to Dan

MALDI-MS Clearly shows the TMPP-66FL mass $C_{43}H_{63}N_5O_{14}P$ 946.401

There are the correct 111 amu pattern ! The post source decay

shows TMPP-66FL, TMPP-66F, TMPP-6C, ! TMPP6

- this was Scrambled. There was a small amount of TMPP-66FL

in the 6-1-1 linker The major product was the 653.24 Low

Corresponding to MASS-66FL This demonstrates the

Successful Light Directed Synthesis of a peptide on a Photo-

patterned Polymer Support ! The MALDI-MS characterization

of the product from the polymer material. It may be

possible to do the MALDI-MS in SITU by Spotting Matrix/TFA

Directly on the plate (Low Res) ! Looking into Mass

Continued on Page

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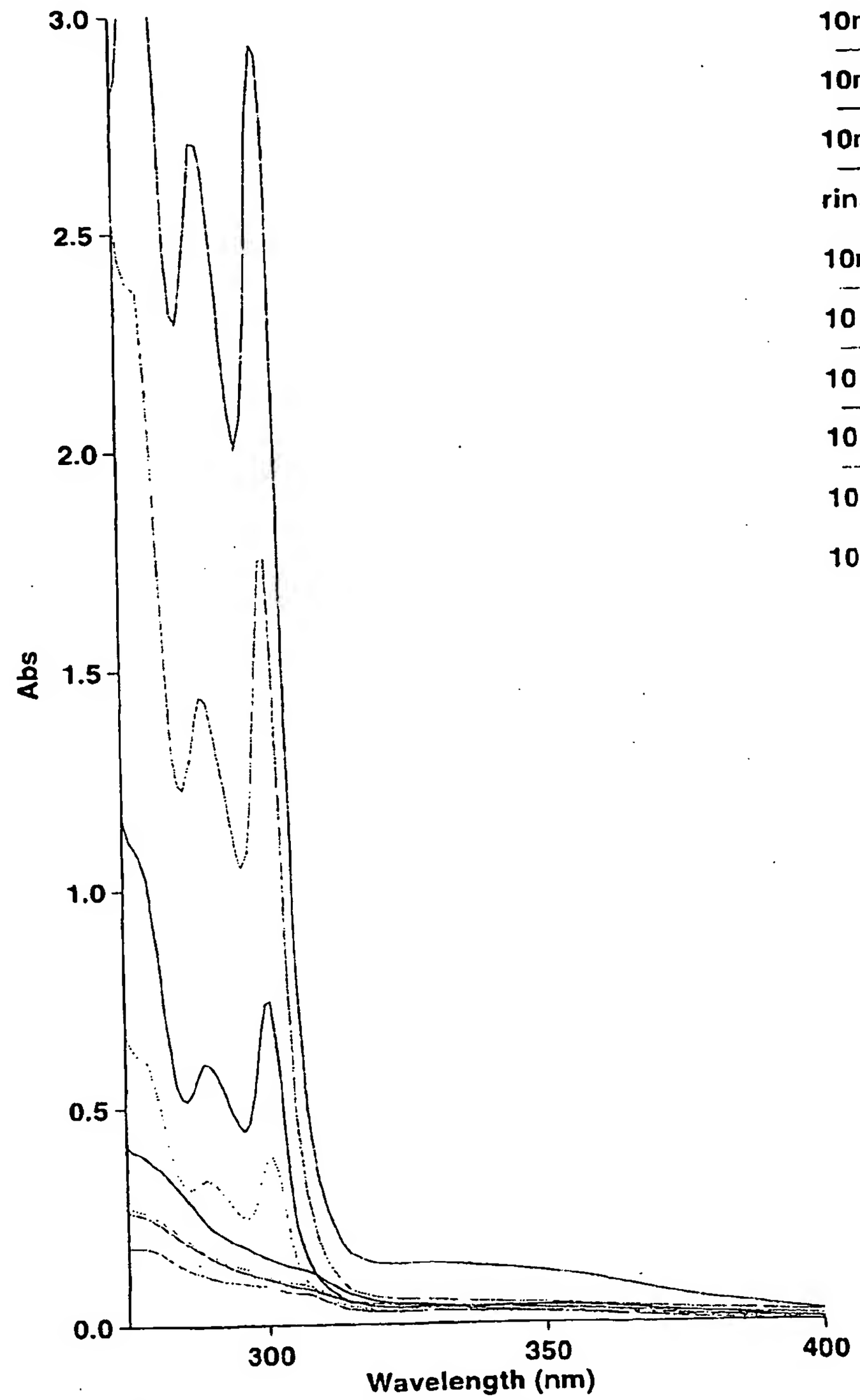
Signed	10.27.04 Date	Signed	10/29/04 Date

[redacted]

PROJECT

Notebook No. _____
Continued From Page 72[73]

9/20/2004 1:25:05 PM Page 1 of 24



10min rinse2

10min piperidine

10min rinse after

rinse after coupl

10min piperidine

10 min rinse afte

10 min deprotecl

10 min rinse afte

10 min rinse afte

10 min piperidin

Signed Date Signed 10/29/04 Date

Purpose: to pattern FMOC-6-FL COOH on the polymer
then Represent w/ MSOC & pattern
P S Y
Hydrolyze Antibody & detect via fluorescence.

From p-x 27 45 nL Hexane + 572 nL Thiol + 4 mg ABN
Washed & Sonicated until dissolved
added to Chamber w/ Fresh Methanol $1.2e
Finished at 2:30 page 29 20x 730/2 8nm FWHM 8mW 25ms 525nm
2x 27x13 arrays, looks great!

Looking for FMOC-6-FL COOH PEPTIDE By RAN. Still
dissolved in TFA/TMS solvent Solvent 1:2:1 - 1.1 = 80 mg
Extracted w/ MeOH (Aq layer w/ (60 nL PIPES in
MeOH unnecessary) Dried down 4hr longer (yellow)
80 mg - 60 nL $\left(\frac{.742\ mg}{\mu L}\right)$ = 35.5 mg peptide
FMOC-6-6-F-L COOH

FMOC 223.3 + 2(57.05) + 147.13 + 113.16 + 17 = 614.74 $\frac{mg}{mmole}$

35.5 mg / 614.74 $\frac{mg}{mmole}$ = 0.058 mmole $\times \left(\frac{2.5\ U}{2.8}\right)$ = 5.5×10^{-2} mmole 11:01
$\times$ 5 = 0.29 mmole DiPEA

(5.5e-2 mmole)(379.25 $\frac{mg}{mmole}$) = 20.9 mg HBTU
(0.29 mmole)(129.25 $\frac{mg}{mmole}$)$\left(1.4\ \frac{\mu L}{mg}\right)$ = 52.5 µL

0.5 mL $\left(\frac{30\ mM}{1000\ mL}\right)$(614.74 mg/mmole) = ~~9.22 mg~~ 9.5 mg p-ptn
then 9.15.04 then 9.15.04

0.5 mL $\left(\frac{30\ mMoles}{1000\ mL}\right)$(379.25 mg/mmole) = $\frac{5.68\ mg\ HBTU}{mL}$
0.0 mL $\left(\frac{60\ mMoles}{1000\ mL}\right)$(129.24 mg/mmole)$\left(\frac{0.742\ mg}{\mu L}\right)$ = 5.2 µL DiPEA
Use 150 mMoles 13. µL

Read and Understood By
Just — Cash

9.15.04
Signed Date

Signed 10/29/04
Date

Continued on Page 72

PROJECT _____ Notebook No. _____

Continued From Page 71

Couple Fmoc-Rink? From page 39 (TN7)

33.73 mg Fmoc-Rink (2.5 eq)

22.5 mg HBTU (2.30 eq)

11.5 µL DIPEA (2.64 eq)

11.5 / 2.64 = 2X / 5

21.7 SCy 2Bn2 ←

+ 0.6mL DMF

Started Rxn @ 2:30 pm 09/15.04 50°C / 150 rpm

3:30 " " Rinsed (3x DMF 2x MeOH) x

10 mM Rink @ 150 RPM OD_{301} = 0.15 Too high

Rinsed @ 50°C in DMF for 10 mM Rink 3x OD_{301} = 0.1

10 mM Rink-NH2 @ 150 RPM OD_{301} = 2.9

Now need to get all the piperazine off

10 mM @ 50°C 150 RPM Rinsed 3x ∴ left overnight in DMF Rinsed 3x w/ DMF OD_{301} = 0.147 Looks like 1st 10 mM Rink after Fmoc-Rink

Couple Peptide Use 1/3 of Peptide Solution. 27 mg Came over to 29 mg ∴ was close to 1/2 of the solution + 6 mg HSTU + 13 µL DIPEA Smushed 5 mM & added to Sulfur @ 910 AM 9/16/04 @ 50°C ; 150 RPM

1:20 PM 9/16/04 Rinsed & mixed 4x w/ DMF

(R) Rinsed @ 50°C Stirring @ 125 PM - 225 PM

(R) Rinsed 3x DMF 20 mM Rink @ 50°C Rink 3x DMF

(R) 10 mM Rink @ RT OD_{301} = 0.11

+ 10 mM 20% Piperazine @ RT 150 RPM OD_{301} = 1.75

(R) Rinsed 3x DMF

+ 14 mg Mac + 40 µL DIPEA 600 µL DMF

50°C 150 RPM 3:30 9/16

Continued on Page 73

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Signed _____ Date 9/16/04

Signed _____ Date 10/29/04

Notebook No. _____ Continued From Page 72[73]

PROJECT _____

Removed from heat @ 440 pm & placed in DH @ RT
(had been in the DH all along)

(N) 920 pm Rinsed 3x in DMF & left overnight

9/16/04

(L) washed 3x DMF 10 min Rock 03,33 =
600µL 60mM Sentaberzin + 20µL Dipet

Experimental Design | 1st 400 Probes to patterned areas

This will be any unreacted Amines & Block the antibody

On these patterned areas

1mJ add ~~territory~~ quitenary areas

730/2 8nm FWHM Z=525µm 20x objective

1mJ for Y
0.1mJ for Y

P@ 2mJ for Both

13 P 720
12 Y 600
11 P 660
10 Y 550
9 P 495
8 Y 440
7 P 385
6 Y 330
5 P 275
4 Y 220
3 P 165
2 Y 110
1 P 55

1 spot 27

Probe Rich 53.96 mg = 337.5% ∴ 21.1 mg Probe Pro
33.73 mg

(P) 3x DMF, 10 min DMF, 3x DMF

Y
P

+ 21.1mg Probe Pro + 225µL H2O + 21.7µL Dilea

50°C 150 Rpm 12:07 pm 9.17.04 - 1:10 pm 9.17.04

(P) Rinsed 3x DMF, 1hr @ 50°C 3x DMF, 1hr DMF

Continued on Page 74

Read and Understood By

Signed 9/7/04 Date

Signed 10/29/04 Date

PROJECT _____ Notebook No. _____

Continued From Page 73

OD_{371} of 10mM Rnase = 0.086

(A) 10mM 20% glucose OD_{301} = 0.742 (Scrubbed w/ Student hours removed... Jay has been on it and they won't recognize

(B) Buffer 300mM 2.9 - 1.75 = 1.15 NOT possible to pipette

$\frac{0.742}{2.9} = 256$ which is the number of factors I detected just

(C) 600mM 60mM Sucrose + 20mM PIPES 6 days 9/27/04

Pipette to 0.1mM for 1st 7 rows (0.1mJ)

UV Electrons @ 1mW & 1sec (1mJ)

(D) 3x Buffer Lower Buffer 3x Buffer

Fructose(RX) at 459.54 g/mole $\frac{539.6}{33.73} = \frac{459.54}{x}$

+ 28.73mg Fructose(RX)ase + 22.5mg HBTU + 21.7μL DMSO + 60mM PIPES

437μm 50°C 150 RPM

(E) Rinse 3x

+ 60μL 60mM Succulence + 20μL DMSO Spin 2min

Pipette after ½ (last Sucr) w/ 2mJ (1sec 2mW)

w/ before.

(F) Rinse 3x Lower @ 50°C

Betaine Monohydrate 99% (Sigma) FW = 135.2

$\frac{539.6}{33.7} = \frac{135.2}{x}$ 8.4mg Betaine

22.5mg HBTU

21.7mL DMSO

Couldn't get betaine to dissolve

Rinse Simple 3x w/ part 1 Continued on Page 75

cut @ 4°C over the weekend

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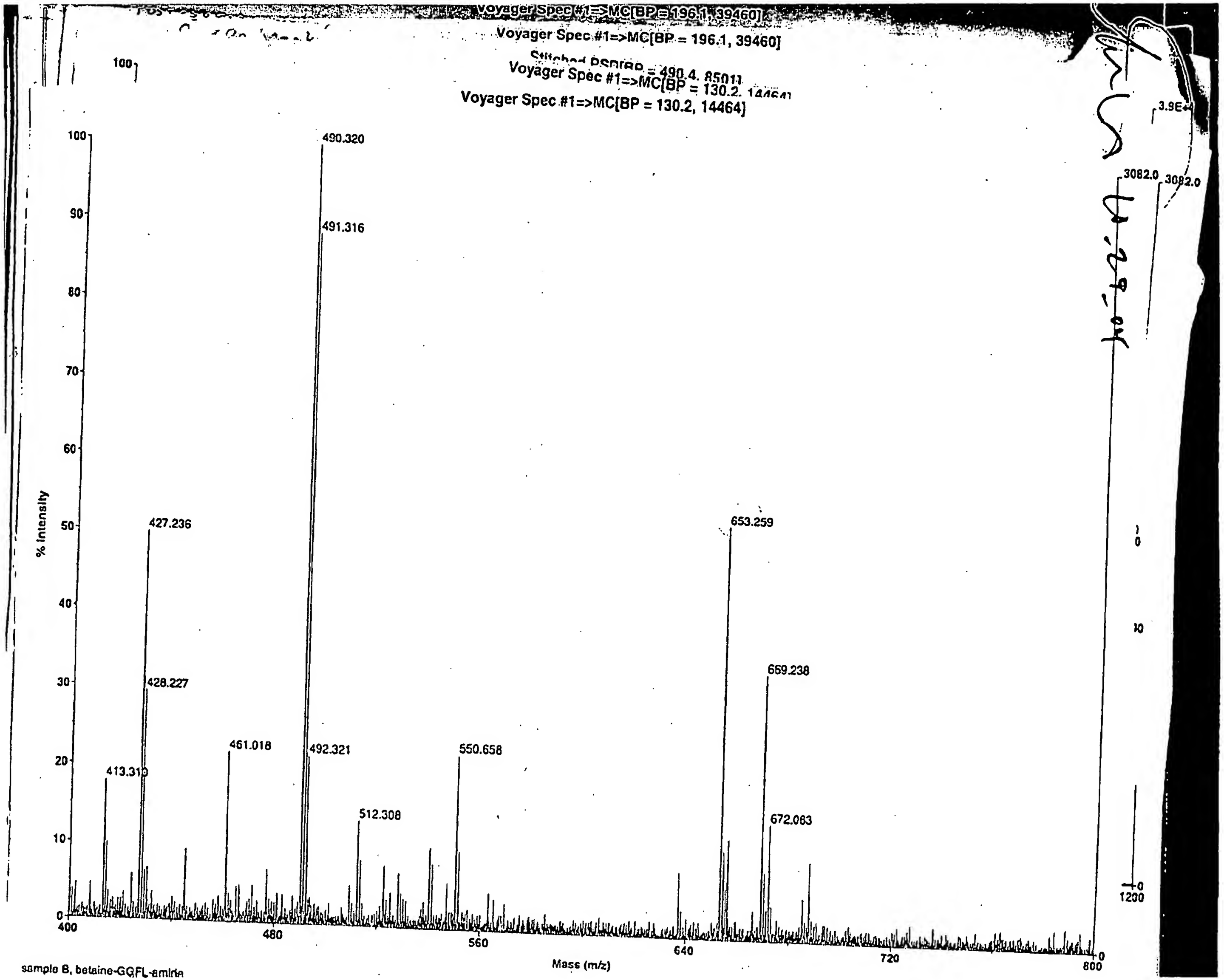
Signed _____ Date 7/17/07

Signed _____ Date 6/29/04


Voyager Spec #1=>MC[BP = 196.1, 39460]
Voyager Spec #1=>MC[BP = 196.1, 39460]
Voyager Spec #1=>MC[BP = 490.4, 85011]
Voyager Spec #1=>MC[BP = 130.2, 14464]
Voyager Spec #1=>MC[BP = 130.2, 14464]
100
90
80
70
60
50
40
30
20
10
0
% Intensity
490.320
491.316
427.236
428.227
413.319
461.018
492.321
512.308
550.658
653.259
669.238
672.063
400
480
560
640
720
800
Mass (m/z)
3.9E+
3082.0
3082.0
sample B, betaine-GGFL-amide

9.20.04 Required 100 mL more to positive < Redo ①

Try 50 mL more rather titrate it, wait

To minimize methyl ester formed.

① 500 mL DMF + 50 Vl MeOH + 8.5 mg Betain + 21.7 µL DiPEA

② + 22.5 µL HBTU

3 min added to chamber reacted @ RT 9.20.04 9:20 am

11:20 am 9.20.04 Rinsed 3x w/ DMF, 1 hr DMF, 10 min Rinse

OD_{309} : 0.086

① Load 2x Repeat OD_{301} = 0.79

⑫ Rinsed 3x DMF 15 min Rinse DMF 3x DMF

Antibody hybridized

3x wash w/ PBS Buffer PH=7.2 30 min

100 µL of L-8516 Lev-conjugated Antibody (Ab3.1)

+ ~~2000~~ 900 mL PBS 2:11 pm 9.20.04 — 3:52 pm.

TW 9.20.04

Rinsed 3x with 0.05% Tween 20/PBS

4 pm ADDED 1/1000 Dilution of Antibody

5 pm rinsed 2x w/ 0.05% Tween in PBS

Mixed w/ 488 nm Incubating 752 SR

Will get the appropriate ratio & would expect

Could be that the antibody binds YP66FL or

Most likely is from the Fluorescent photoproduct.

Conclusion | The most interesting part so far is that I can

get ~100% yield @ (2mJ) I seem much more

fluorescence when I go for 100% w/ (5mJ) Next

Read and Understood

for more info

Continued on Page 76

9/17/04 10/29/04

Signed Date Signed Date

Scraped off the two halves of the resin pellet
& placed the polymer particles into separate microfuge
tubes added 200µl 50:50 TFA in DCM w/ 3% Triisopropylsilane
10:35am 9.21.04
1:30pm still yellow ~~added~~ removed solvent & dried down
Observations the fibers are yellow, both for the particles &
Control. I know they will become clear after the cleavage?
did not change in 1:1 DCM:TFA
1:30pm added neat TFA & immediately the
fibers lost their color after small
particles added 0.3µl Triisopropylsilane
→ not sure, polymer floated in TFA so it
was dissolved so I GUESS see the color
added could accurately & could see color
did burn à green & one fiber no's

9/22/04

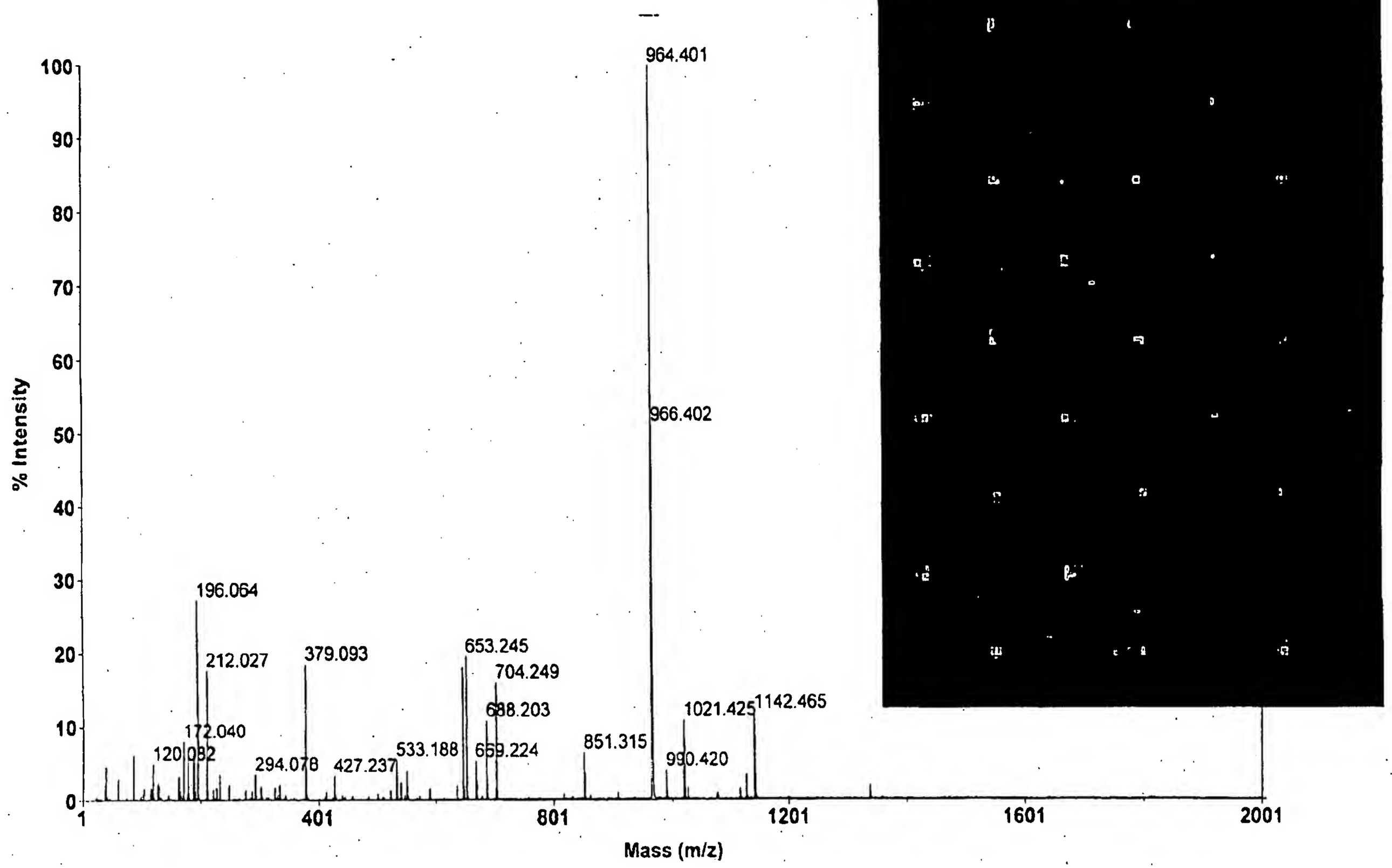
I ran 9/23/04

→ Repeated cleavage on 2 beads of B-66FL-Amide
(N one bead NVOC-66FL-Amide
in 12 Triisopropylsilane in TFA
Used two assay (66FL & YP66FL) in 1:1
TFA: Acetonitrile w/ 0.5% Triisopropylsilane (Same cocktail
the polymer may have fallen apart,
MALDI-MS shows m/z = 440.32 peak which is 1 Da off from predicted product
structure Control shows m/z = 653.259 : NVOC 66FL-Amide
Ran 10/29/04

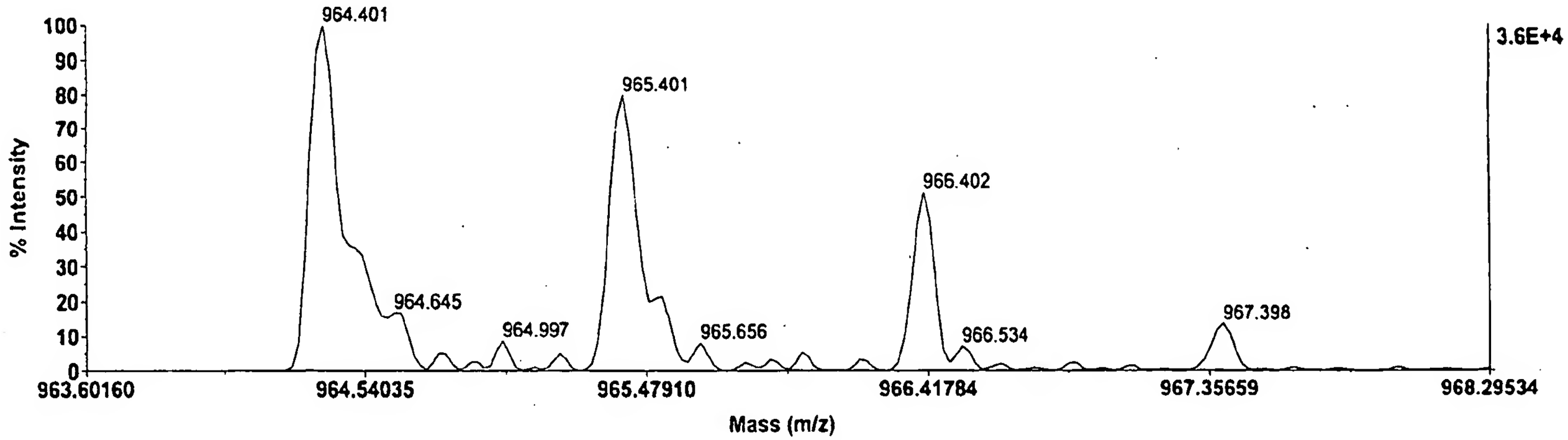
Continued on Page

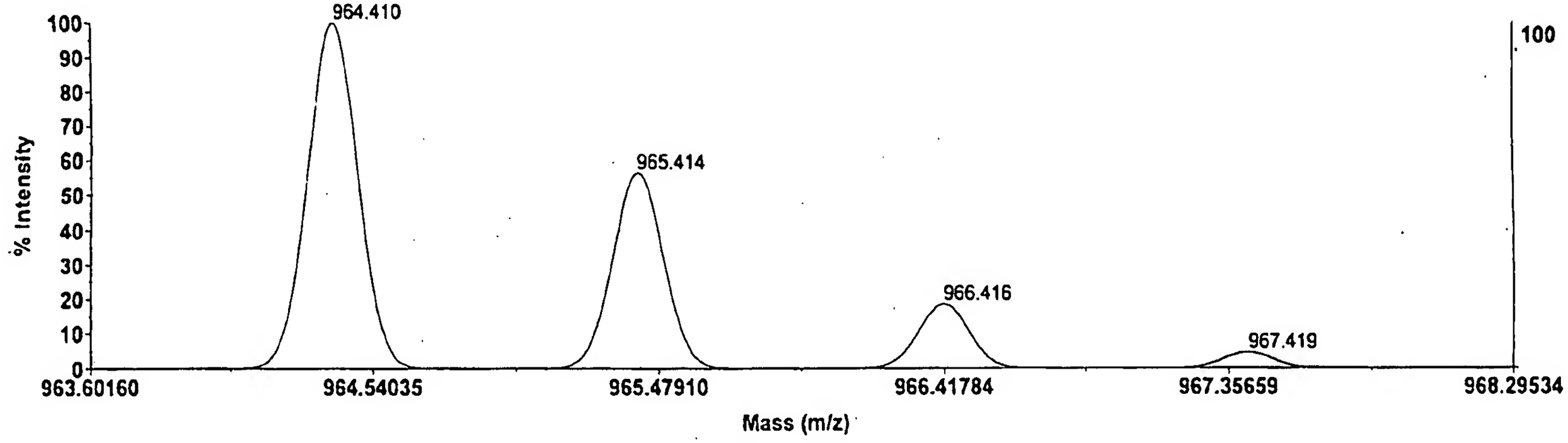
Read and Understood By

Signed _____ Date 9.22.04 Signed _____ Date 10/29/04

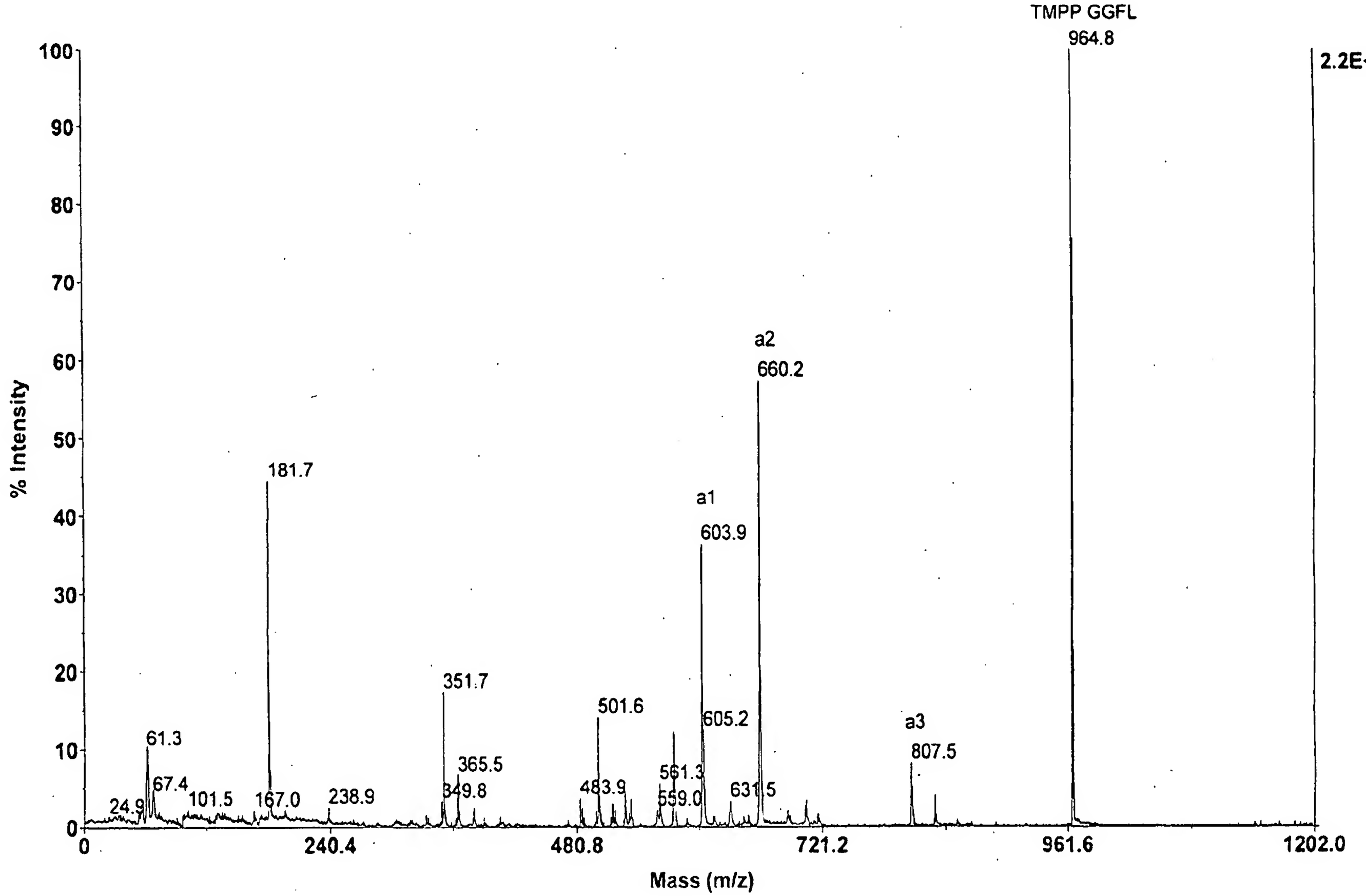


Calibrated MALDI-TOF MS spectrum showing ions formed from photopatterned *N*-Tris(2,4,6-trimethoxyphenyl)phosphine-GGFL (m/z=964.4 Da) peptide. Inset image of photopatterned array of texas red sulfonyl chloride (Red) and fluorescein isothiocyanate (green). *N*-Tris(2,4,6-trimethoxyphenyl)phosphine (TMPP) is facilitates product detection and formation of a ions for post source decay analysis. Analytical Biochemistry 268, 305-317 (1999).

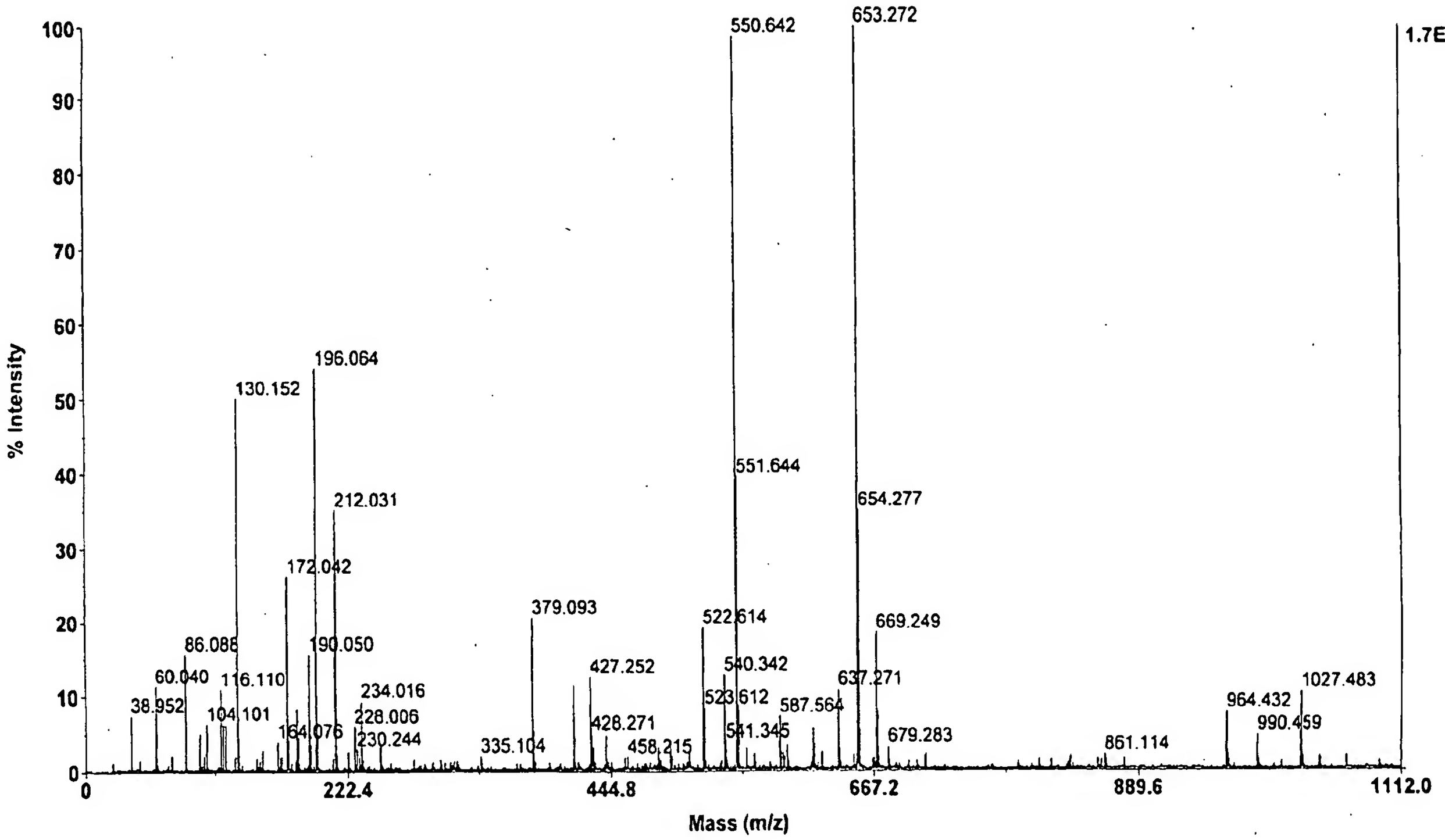




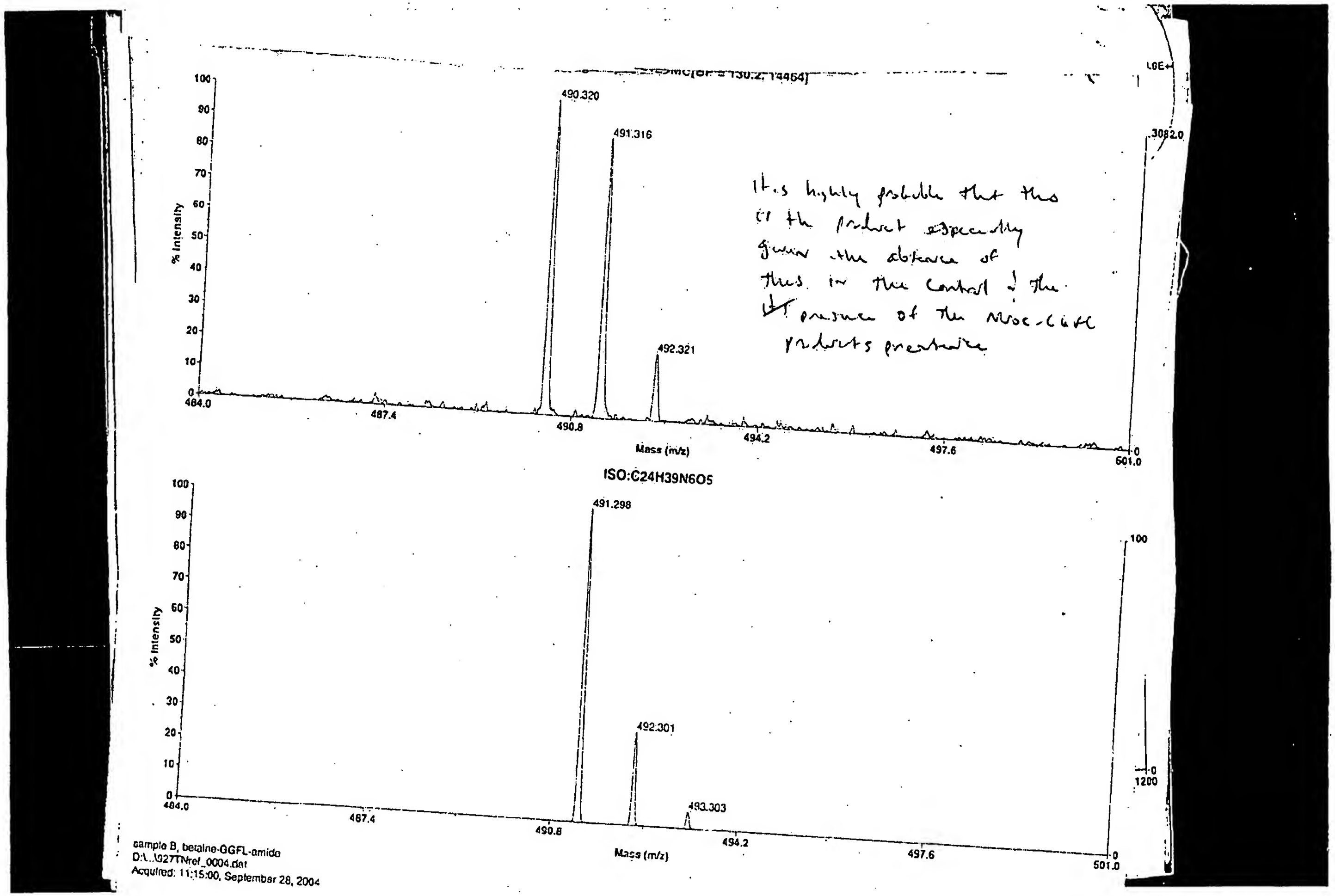
Calibrated MALDI-TOF MS spectum of observed isotopic distribution for the m/z=964.4 Da ion vs. those predicted for the TMPP-GGFL [C48H63N5O14P] (bottom) .

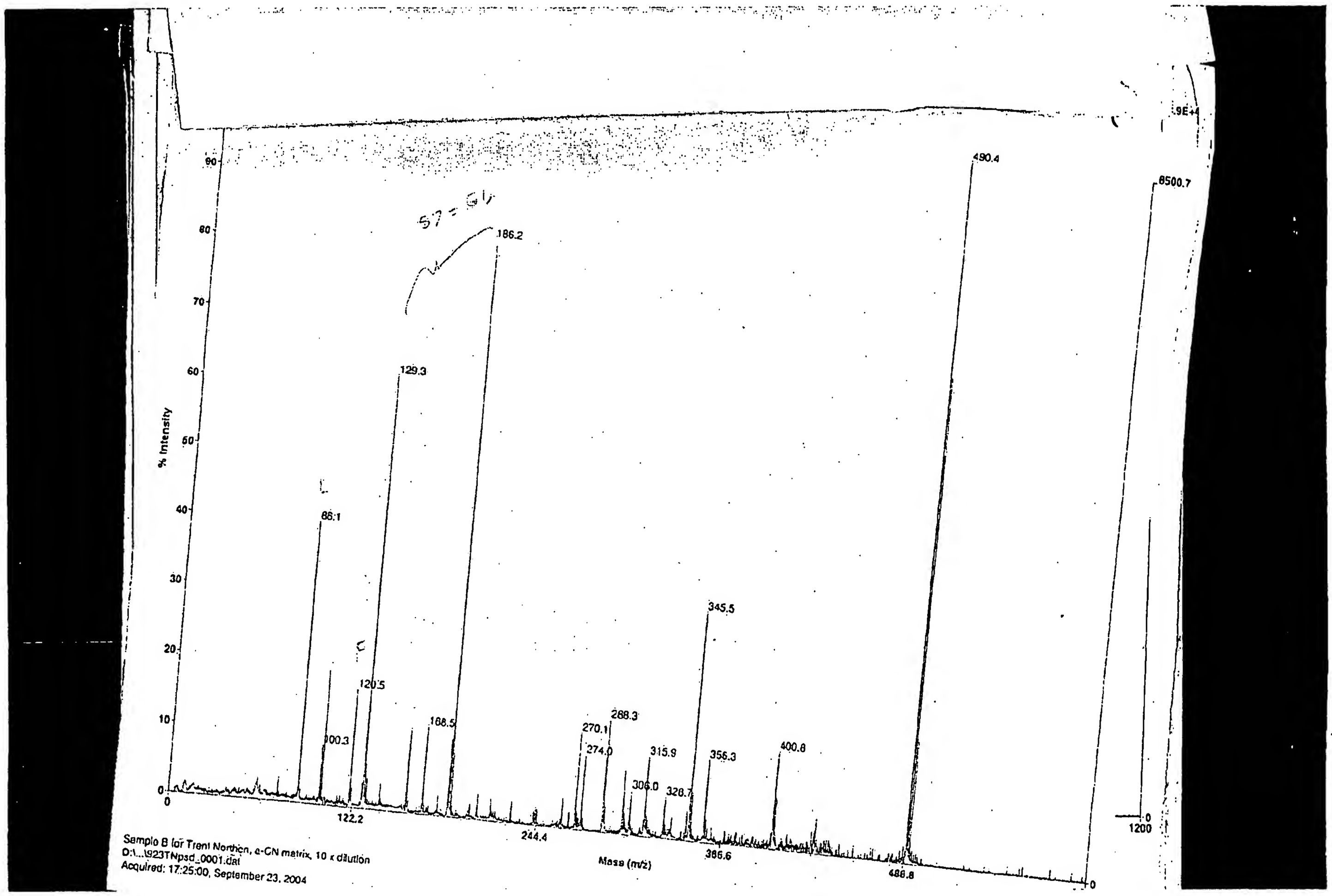


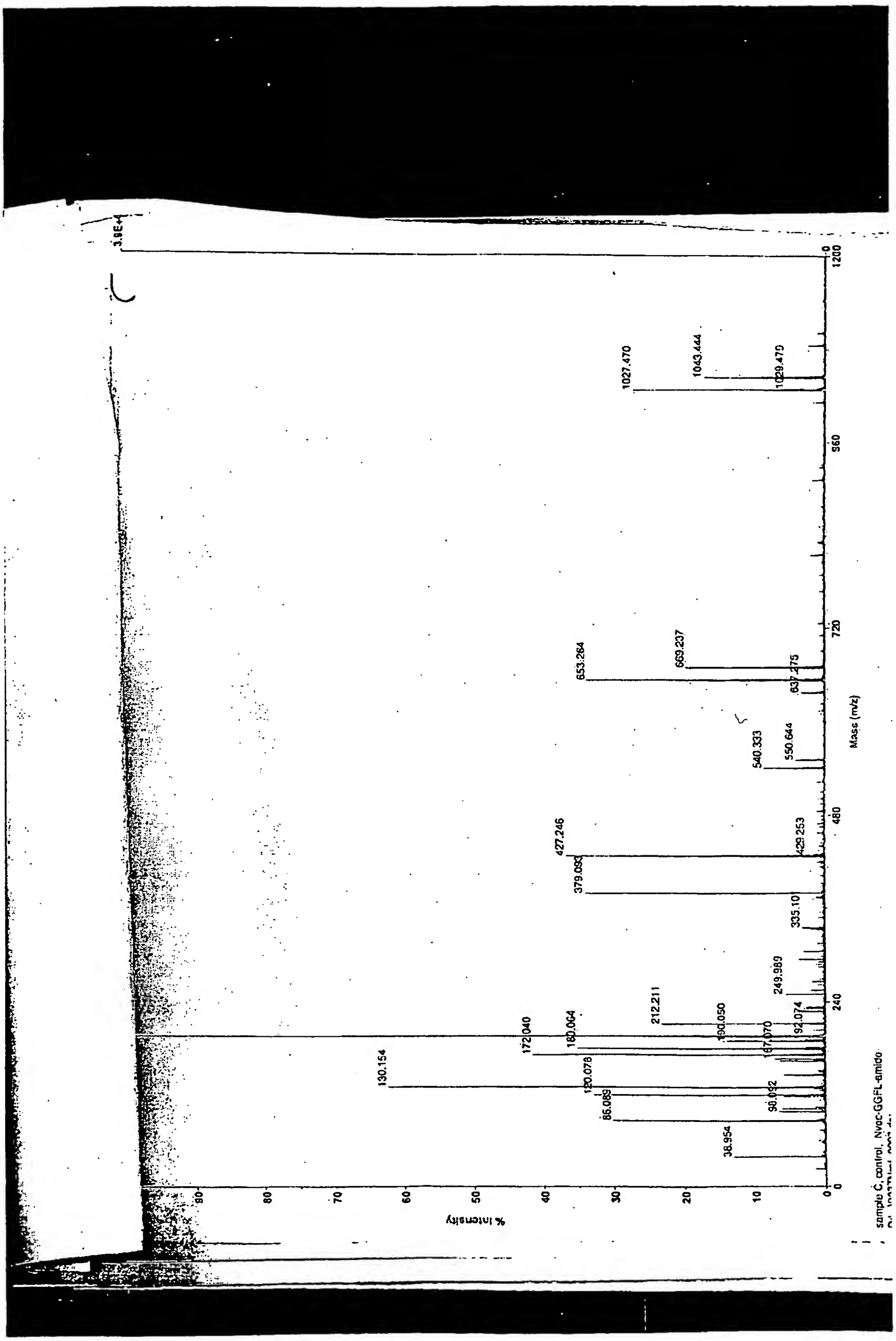
Uncalibrated MALDI-TOF MS post source decay showing the a1 (TMPP-G), a2 (TMPP-GG), a3 (TMPP-GGF), and primary ion m/z=964.8 Da of the TMPP-GGFL peptide.



Calibrated MALDI-TOF MS spectrum showing ions formed from control (not irradiated) areas.


490.320
491.316
492.321
% Intensity
Mass (m/z)
484.0
487.4
490.8
494.2
497.6
601.0
3082.0
LOE
It is highly probable that this is the product especially given the absence of this in the control & the presence of the Moc-GGFL products previously
ISO:C24H39N6O5
491.298
492.301
493.303
sample B, betaine-GGFL-amide
D:\...\927TNref_0004.dat
Acquired: 11:15:00, September 28, 2004
501.0
1200


% Intensity
Mass (m/z)
86.1
120.5
129.3
168.5
186.2
300.3
270.1
274.0
288.3
306.0
315.9
328.7
345.5
356.3
400.8
490.4
488.8
6500.7
9E+4
122.2
244.4
386.6
0
1200
Sample B for Trent Northen, α-CN matrix, 10 x dilution
D:\...\923TNpsd_0001.dat
Acquired: 17:25:00, September 23, 2004


3.9E+4
0
1200
1027.470
1043.444
1029.479
960
669.237
653.264
637.275
720
540.333
550.644
Mass (m/z)
427.246
379.089
429.253
480
335.10
249.989
212.211
190.050
187.070
172.040
180.004
130.154
120.078
92.074
96.092
86.089
38.954
240
0
% Intensity
sample C, control, Nvoc-GGFL-amido

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

ATKINS, Robert, D.
Quarles & Brady Streich Lang, LLP
One Renaissance Square
Two North Central Avenue
Phoenix, AZ 85004
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 19 July 2005 (19.07.2005)	
Applicant's or agent's file reference 112624.00138 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US2005/015764	International filing date *(day/month/year)* 06 May 2005 (06.05.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 06 May 2004 (06.05.2004)
Applicant ARIZONA BOARD OF REGENTS, acting for and on behalf of, Arizona State University et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)***An asterisk (*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No	Country or regional Office or PCT receiving Office	Date of receipt of priority document
06 May 2004 (06.05.2004)	60/569,370	US	20 June 2005 (20.06.2005)
10 September 2004 (10.09.2004)	60/608,774	US	20 June 2005 (20.06.2005)
29 October 2004 (29.10.2004)	60/623,181	US	20 June 2005 (20.06.2005)

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1211 Geneva 20, Switzerland

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Telephone No. +41 22 338 8147

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